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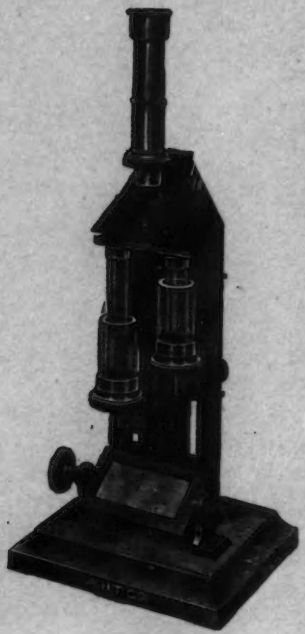

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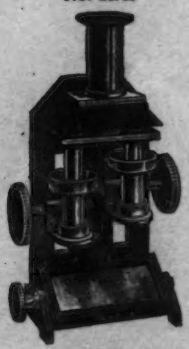

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THE AMERICAN
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VOL. 42 FEBRUARY 1, 1917 No. 3

RESEARCHES ON THE EXCHANGE OF ENERGY IN LIVE ANIMAL TISSUES

II. Study on the Liver

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I. INTRODUCTION

The technical difficulties surrounding the obtaining of exact and direct measurements of small amounts of heat developed in large periods of time, have prevented the conclusion of the chapter of physiology on animal heat (which, as regards the animal itself, whole and intact, has progressed considerably and is at present in a high stage of perfection), as such a conclusion depends on the analysis of each organ, and each separate tissue, of the system to which they belong, in order to determine, once for all, the rôle of each organ or tissue in the total production of heat. These difficulties, unsurmountable until quite recently, on the one hand, and the importance of the problem on the other, induced physiologists to attempt measuring the heat produced by each organ, and, in many instances, they were content with just an idea, more or less approximate, of this production, obtained by indirect and extremely defective methods. They had recourse, in some cases, to mere thermometrical readings; and, in others, to the valuation of the amount of heat generated, using as a basis the amount of O_2 absorbed or of CO_2 exhaled.

The first method may lead us to admit, at the most, that an organ which becomes much heated (as a muscle in operation), or the temperature of which is maintained above that of its environment (as happens with the liver), also generates much heat; such a method

however, does not make it possible to determine absolutely the amount of heat generated. The second method would appear, on a cursory analysis, to be satisfactory. It comprises a double operation: (1) the determination of the amount of respiratory exchanges; and (2) the indication of the thermical coefficients of CO_2 and O_2, by which should be multiplied the figures obtained by the first operation, in order to ascertain the heat generated.

The respiratory exchanges of an organ left "in situ" and with all its relations (nervous, vascular and so forth) intact, could be determined by measuring the total amount of gases in the blood before and after passing through such organ; in view, however, of the uncertainty of blood irrigation, which constantly varies, and the equal oscillation of the intensity of respiration itself, this operation would require continuous, or almost continuous, sampling of arterial and venous blood and, furthermore, the exact knowledge, during the experiment, of the total quantity of blood which passed through the organ. The mere statement of these conditions shows us the difficulty—one may almost say the impossibility—of obtaining precise results. But, even admitting that it were possible to obtain the exact amount of O_2 and CO_2 absorbed by the organ under experiment, the fact that all its organic (nervous, vascular, etc.) connections were intact, might occasion a mistake in the valuation of the heat generated, as the total quantity of chemical energy released by the absorption of O_2 may not be transformed, directly and immediately, into heat but into another form of intermediary energy which, passing on to another organ, would then be turned into heat. Let us suppose, for instance, that a part of the chemical energy supplied to the cerebrum by the combustion of food, is released by it for the purpose of generating impulses which are passed on to the muscles; the valuations of heat, of the cerebrum or of the muscles, taking as a basis the respiratory exchanges, would bring us to figures which would be excessive for the cerebrum and too small altogether for the muscles. These hypotheses may not become true, but the mere possibility of their occurring is sufficient to cause mistrust and doubt of the value of respiratory calorimetry under such conditions, and makes it imperative for us, in order to simplify the study of the matter, to work with isolated organs, separated from all and any organic connection.

Once the value of respiratory exchanges is known, that is to say, once the quantity of O_2 consumed, and of CO_2 generated, have been ascertained, it is necessary to multiply one of these numbers (let us

say O_2, for instance) by its thermical coefficient.[1] But, even then, in order that the amount of heat calculated may be as near the real figure as possible, it is advisable to determine the nature of the alimentary substances burned; this problem is solved by respiratory calorimetry applied to an animal, taking into account, during the experiment, its condition of nourishment or fasting, the nature of the food absorbed (protein, fats or carbohydrates), the respiratory quotient of Pflüger. When, however, one is dealing with isolated tissues, these indications of the nature of the food potentials burned lose their significance and all that remains is the respiratory quotient, which, by itself, does not enable one to determine the nature of the food, because its present value may be the result of the simultaneous combustion of various substances. Then, again, one knows that the animal receives food always in the same initial condition (protein, fats, and carbohydrates) and abandons them in the same condition—CO_2, H_2O and urea—or bodies very much the same from a thermo-chemical point of view; the amount of heat generated by the transformation of one molecule of each of these substances depends, therefore, on its initial as well as on its final condition, and the respective thermical coefficients of O_2 are also an exclusive function of the said initial and final conditions besides depending, of course, on the very nature of the substance transformed. Now, between the initial condition and the final condition of one of these foods, many intermediary phases occur, the majority of which are, as yet, unknown. If, therefore, these phases occur in different organs, it follows that the thermical coefficients will also vary from organ to organ, and thus the respiratory calorimetry applied to isolated parts of the organism would induce one into ever increasing error. For instance: If the total transformation of fat into CO_2 and H_2O by combustion, takes place, as is most likely, in two phases: the first of the fat into glycose in the liver and the second, from glycose into CO_2 and H_2O in other tissues, as for instance, in the muscles, the amounts of heat developed by each of these phases will be different from each other, and the thermical coefficient to be used in each case will be that of fat for the entire organism, that of glycose for the muscles, and that of the transformation of fat into glycose for the liver, on the value of which there are still some doubts.

[1] The thermical coefficients of O_2 instead of those of CO_2 are recommended because the thermical coefficients of O_2 have the great advantage of being much less variable according to the nature of the food burned.

In the above example, however, we have taken it for granted that the initial and the final conditions of the transformation in each organ are already known, and, of course, if they are not known, the uncertainty will be but increased. In short, one may say that just because respiratory calorimetry may render it possible to calculate the amount of heat generated by an animal, it does not follow that it is also possible to calculate thereby the amount of heat generated by each organ or each tissue of the said animal; the problem, in the latter case, is indefinite, and the probabilities of error in these indirect respiratory valuations are so many that more direct measures are indispensable.

In this paper we purpose: (1) Measuring the amount of heat developed by the liver; (2) measuring its respiratory exchanges; (3) establishing the value of the thermical coefficients of O_2 and CO_2 for the liver.

STUDIES ON THE LIVER

We have made use of the technique studied in our preceding paper (1). As we then saw, the tissues are reduced to small fragments, so as to considerably increase their surface of respiratory exchange with their environment. This is the application of the principle used by Battelli and Stern in their experiments on the respiration of tissues. These authors (2) demonstrated that, even after total destruction of the cellular structure, the fragments of cells and the liquids from same were still able for some time to absorb O_2 and eliminate CO_2. It is easy to understand, however, under conditions so entirely abnormal, the results obtained could hardly be applied to the case of normal organs; it was, therefore, necessary for us to make sure that our grinding apparatus would respect the integrity of the great majority of the cells, acting as a mere mechanical separator of the organ; to make sure of this, which otherwise might eventually prove an objection to the technique adopted, we asked Prof. Raul Leitão de Cunha, teacher of anatomy and pathological physiology of the Rio de Janeiro Academy of Medicine, to make a microscopic examination of the liver emulsions used by us.

The following is the report of the Professor, to whom we are exceedingly grateful:

The histological analysis of the matter submitted resulted in the identification or tracing of the following elements:

(1) A large number of deformed blood corpuscles (hematias) which, in view of their number, constituted the predominant element.

(2) Hepatic cells distributed according to the following types:
  (a) Detached and well shaped, as regards nucleus and protoplasm.
  (b) Together in pairs, and in the same morphic conditions as above.
  (c) In single file, well shaped, with up to six cells per string.
  (d) Grouped in cords of two or three strings (as described in letter c) joined together.
  (e) Forming large spots or plates, but one layer deep thus allowing the verification of their anatomic integrity.
  (f) In more or less voluminous masses, in which their connections with each other are maintained intact.
  (g) Detached or agglomerated, but broken up into fragments more or less difficult to identify.

(3) Groupings of leucocytes, mainly polymorphous-nucleated.

(4) Remainders of the Glisson capsule, joined in masses of various sizes.

(5) Vascular débris and others, forming amorphous agglomerates, difficult to penetrate with the light of the microscope.

It is evident from the above that the liver emulsion used in the experiments which we shall proceed to describe contains, as a rule, cells either completely detached or in groups small enough to prevent asphyxiation and to allow their easy respiration.

The following table condenses the result of the experiments made on the liver in pursuance of the technique mentioned before.

Table showing the result of experiments made on liver emulsion

ORDER NO.	CO_2	O_2	$\frac{CO_2}{O_2}$	HEAT GENERATED (Q)	THERMICAL COEFFICIENT OF $O_2 = \frac{Q}{O_2}$	THERMICAL COEFFICIENT OF $CO_2 = \frac{Q}{CO_2}$
Experiment I.....	347.4	330.0	1.05	2639.2	7.99	7.59
Experiment II.....	468.2	418.0	1.12	2633.2	6.29	5.62
Experiment III.....	529.6	436.0	1.21	3370.6	7.72	6.36
Experiment IV.....	353.6	303.4	1.16	2306.0	7.60	6.52
Experiment V.....	506.1	390.0	1.29	2253.0	5.77	4.45
Experiment VI.....	355.8	324.9	1.09	2099.0	6.46	5.89
Average.....	426.7	367.0	1.15	2550.1	6.97	6.07

NOTE. CO_2 and O_2 are reduced when dry and at a pressure of 760 and 0°; the figures shown in the above table represent cubic centimeters and refer to the respiratory exchanges of 1 kilo of liver during one hour.

The heat is represented in gram-calories per kilo-hour.

The results obtained are quite different from what might be expected. The thermical coefficients of O_2 and CO_2 can not be connected with any of the coefficients relative to the common alimentary substances—

protein, fats or carbohydrates, and even less to the thermical coefficients of transformation of the nutritive reserves, which develop a lesser amount of heat. Furthermore, if the average of the coefficients of O_2 (6.97) is greater than the thermical coefficient of all the transformations known to take place in the organism, it is also too great for any of the intermediary phases. We are, therefore, led to admit either that the amount of heat determined by us is excessive and wrong, or else that the measurement of the gases resulted in figures altogether too small for O_2. We are positive that the measurements of heat are rigidly conducted and that the results really represent the actual facts, as may be verified by our first paper, and by a thorough discussion of the method employed. As to the measurement of the gases, however, we must confess to great doubt as to their accuracy. Compared with the figures of Battelli and Stern (3), the absolute values of our readings are lower, but this is probably due to the fact that, in their experiments, these authors submitted the tissues to a much more energetic agitation than we did, and also to the fact that the tissues breathe in an atmosphere of pure oxygen, and not in the common atmospheric air. We have, indeed, remarked that, towards the end of each experiment, the emulsion of tissues in defibrinated blood presents a more or less dark or venous color, although there are still notable quantities of O_2 in the atmosphere of the bottle, which would indicate slowness in the diffiusion of O_2 and of its absorption by the tissues. On the other hand, however, the value of the respiratory quotient $\frac{CO_2}{O_2}$ is similar to that obtained by Battelli and Stern, for the liver.

In our experiments, as in the majority of the experiments of these authors, the relation $\frac{CO_2}{O_2}$ has very high values, exceeding unity. Now, were this true it would mean, most assuredly, a transformation of carbohydrates into fats, in the liver, detached or isolated, an extremely interesting thing, but one which is neither plausible nor true, as we shall later demonstrate. Comparing this with the exclusive value of the thermical coefficient of O_2, we are led to admit that our measurements of the gases, taken as they were, notwithstanding, with considerably more care and rigidness than by the gross and inexact methods of Battelli and Stern, yet contain causes of error which it would be advisable to obviate. With this in view, we have originated the following technique for the measuring of gases.

PROCEDURE FOR MEASURING RESPIRATORY EXCHANGE OF TISSUES

Up to now, in endeavoring to determine the proportion of the respiratory exchanges of tissues, we used the following procedure: A sample was taken of the air in the calorimeter and submitted to eudiometric analysis, the carbonic gas then found, or the deficit of O_2 registered, being considered by us as representing faithfully the respiratory exchanges of the tissues, just as if they had breathed directly in the air. This is the method followed by Battelli and Stern in many of their experiments; in others, however, where the tissues breathed in an atmosphere of O_2, a simple manometric measurement showed the decrease in the volume of O_2 in the breathing chamber and, consequently, the amount of O_2 absorbed.

We shall now consider the tissue breathing in defibrinated blood, or even in the suspension of red globules in Locke's fluid, which we have designated as "artificial blood." The oxygen consumed by the tissues is absorbed from the O_2 contained in the artificial blood, in dissolution or combined with the hemoglobin of the red globules; as the O_2 in the artificial blood diminishes in consequence of this operation, a new quantity of O_2, drawn from the gaseous atmosphere of the calorimeter, is then absorbed. There is, however, as is well known, no simple relation of equality between the amount of O_2 consumed by the tissues and the amount of O_2 drawn from the atmosphere of the calorimeter, no matter how intensely the tissues are agitated. On the other hand, the CO_2 in the air of the breathing chamber represents the addition of two parts: one produced by the tissues during the experiment, and the other (which is most variable in volume) already existing in the tissues and in the defibrinated blood when placed in the calorimeter. One may, therefore, state that the variation in the composition of the atmosphere of the calorimeter does not determine the amount of respiratory exchanges during the experiments.

To attain this end, it is necessary to proceed, in each case, to two measurements of the gases, one at the beginning, and one at the end of the experiment, so as to obtain, by difference, the real value of the respiratory exchanges; furthermore, each operation should be so made as to determine simultaneously the amount of the gases in the atmosphere of the calorimeter and that of the gases combined or dissolved in the emulsion of tissues. The problem is, therefore, rather a complex one and, to simplify it, we release the gases of the emulsion, add them to the gases which form the atmosphere of the calorimeter, and pro-

ceed with the operation for determining the totality of gases of this mixture, which represents the entire quantity in experiment. To obtain the gases in the blood and in the tissues, the emulsion is submitted to treatment by a 20 per cent solution of phosphoric acid, to free the CO_2 and by a solution of ferri-cyanid of potassium, less than two months old, to free the O_2 by transformation of hemoglobin into methemoglobin. In other words, we use Haldane's method of chemical analysis of gases of the blood (4). Our experiment made it clear that these two solutions could be mixed and used simultaneously; the transformation of hemoglobin into methemoglobin by the ferr -cyanid solution takes place even more rapidly w th the addition of phosphoric acid, than without it, and, on the other hand, this chemical substance absolutely does not prevent the release of the CO_2 in the blood by the

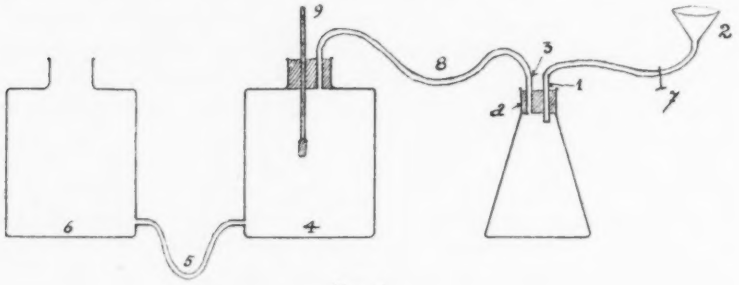

FIG. 1

phosphoric acid. This above method does not give absolute value of the quantities of gas; but, in a comparative analysis, the results obtained by difference are extremely precise.

To apply to our case the general principles of chemical analysis of the blood gases, we originated the technique which we shall proceed to describe. The emulsion of tissues is prepared in double the quantity required for the calorimetric experiment; it is then divided into two equal parts, brought to the same temperature (see memo.) and, at the right moment, a part is poured into the calorimeter for the experiment, and the other is placed in an Erlenmeyer bottle, of the same capacity as that of the calorimetric receptacle. This bottle has a stopper, (*a*) through which pass tube (*1*) connected with funnel, (*2*), and tube (*3*), connected by a rubber tube, (*8*), to receptacle, (*4*), which, in turn, is connected by a rubber tube, (*5*), to receptacle (*6*). Receptacles (*4*) and (*6*) form, therefore, a system of intercommuni-

eating vessels, in which is placed mineral oil (which will not absorb the gases) in such quantity that, when receptacle (*6*) is raised conveniently, the oil will completely fill receptacle (*4*), this being their initial posit on, and so that, when both receptacles are placed on the same horizontal plane (in such a way that the level of the liquid in each bottle will be equal) the volume of the gases in receptacle (*4*) will be known and constant, equal, for instance, to 1000 cc.[2] The emulsion of tissues having been placed in the Erlenmeyer bottle, this is closed with stopper (*a*), the pressure cock (*7*) is opened and the mixture of ferricyanid and phosphoric acid contained in funnel (*2*) is allowed to flow down. During all this time, the bottle should be constantly agitated. These liquids, besides releasing the gases of the emulsion, will immediately cause its respiration to cease, not only on account of the powerful acidity they will originate, but also in view of the toxic action of the ferri-cyanid of potassium.

The excess of gases will then begin to pass into receptacle (*4*), reducing the level of the oil contained therein. When all the gases of the emulsion are released (say in ten minutes),[3] the remaining gases, contained in the Erlenmeyer bottle, are forced out by merely filling the bottle completely with mineral oil through funnel (*2*). Then, with receptacles (*4*) and (*6*) on the same horizontal plane, rubber tube (*8*) is withdrawn from glass tube (*3*), and the latter is allowed to communicate with the atmosphere.[4] When this is done, the oil in receptacle (*4*) will fall until it is on the same level with the liquid contained in receptacle (*6*), whilst, at the same time, it will draw in a certain amount of air from outside.

We shall then have in receptacle (*4*) a known volume of air, equal, for instance, to 1000 cc. at atmospheric pressure and at the temperature registered by the thermometer (*9*). This volume of air will contain all the gases of the emulsion of tissues, plus an unknown quantity of atmospheric air, (part, air already contained in the Erlenmeyer bottle, and, part, air drawn in from the atmosphere). When the calorimetric experiment is finished, the same operation is repeated for the suspension of tissues, substituting the Erlenmeyer bottle by the calori-

[2] This volume should considerably exceed that of the calorimetric receptacle, or that of the Erlenmeyer bottle.

[3] We always allow the same length of time for the release of the gases from the emulsion—ten minutes, during which time the bottle is strongly agitated.

[4] As tube (*8*) is a long thin tube, and during this phase of the experiment the pressure in receptacle (*4*) is less than the atmospheric pressure, there is no fear of losing the gases contained in receptacle (*4*).

metric receptacle, the stopper of which, as we have already seen, is also crossed by two glass tubes. Thus we have a second gaseous mass, modified in its composition by the respiration of the tissues, during the calorimetric experiment. We then make an eudiometric analysis of these two gaseous volumes, which we may call:

(A) The first, or the one indicating the amount of gases in the tissues, when they were placed in the calorimeter; and

(B) The second, showing those found at the conclusion of the calorimetric experiment.

The results, when dry, are reduced, at a pressure of 0°, 760 mm. and with these data we are able to determine the quantities of O_2 and CO_2 breathed during the calorimetric experiment, as we shall proceed to demonstrate.

By the first analysis (A), we obtain the total quantity (Q) of O_2 existing in the said volume; (Q) being equal to the sum of two items, namely, the first (Y_1) representing the volume of O_2 originally existing in the Erlenmeyer bottle, and in the air drawn in from the atmosphere by receptacle (*4*), to complete the final volume of (A), as stated above; the second (Y_2), representing the volume of O_2 existing in the emulsion of tissues, either in dissolution, or combined with the hemoglobin of the blood.

We have, therefore:

$$Q = Y_1 + Y_2 \qquad (A)$$

As (Q) is known, from the eudiometric analysis, if we also knew (Y_1) we would establish, by difference, the quantity which we are endeavoring to ascertain (Y_2).

By the second analysis (B), we find that the quantity (Q′) of O_2 measured, is equal to the quantity (X_1) of atmospheric O_2 which existed in the calorimeter at the beginning of the experiment, or which was drawn in by receptacle (*4*), plus the quantity originally existing in the emulsion of tissues, deducting from the total of this addition the amount of O_2 breathed by the tissues (X_2) during the experiment. We have, therefore:

$$Q' = X_1 + Y_2 - X_2 \qquad (B)$$

By this system of equations, (Q) and (Q′) are obtained by the experiment, and (Y_1) and (X_1) may be calculated if we take as a basis the following observations:

In the first place: Y_1 and X_1 (the O_2 being drawn in from the surrounding atmosphere, or having entered, from the atmosphere, into the calo-

rimeter or into the Erlenmeyer bottle) are accompanied by such a quantity of nitrogen that the relation $\frac{O_2}{N}$ is constant; thus, in the open air, this relation is equal to 0.264 and holds true with remarkable stability even in the most variable climates; in our laboratory, where the windows are always open into a garden, this relation is constant; however, if the operations are conducted in a confined atmosphere, there is no reason why the necessary steps should not be taken to determine the value of $\frac{O_2}{N}$ in the atmosphere where the experiment is made.

In the second place: nitrogen is chemically indifferent to reactions which take place in the tissues, and its quantity remains unchanged. Now, analyses (A) and (B) indicate the quantities (N) and (N′) of nitrogen contained therein, and the quantities (Y_1) and (X_1) of O_2 are, therefore, given by the following expressions:

$$Y_1 = 0.264N$$
$$X_1 = 0.264N'$$

Substituting these values in the equations (A) and (B), we obtain the following system of two equations with two incognitas:

$$Q = 0.264N + Y_2 \qquad (A)$$
$$Q' = 0.264N' + Y_2 - X_2 \qquad (B)$$

taking the value of Y from (A) and substituting in (B), we have:

$$Q' = 0.264N' + Q - 0.264N - X_2$$

therefore:

$$X_2 = 0.264(N' - N) + (Q - Q')$$

N and N′ are the volumes of nitrogen, and Q and Q′ are the volumes of O_2 determined in (A) and (B).

Therefore, the quantity of O_2 breathed by the emulsion of tissues is equal to the product of the relation between the O_2 and the nitrogen in the air of the laboratory, (as a rule 0.264) by the difference between the volumes of N determined in (B) and (A), plus the difference between the volume of O_2 determined in (A) and (B).

The nitrogen originally existing in the emulsion of tissues does not affect the results obtained, because its quantity, which depends exclusively on the temperature of the emulsion and on the pressure of the nitrogen in the outside air, varies but little, and is constant in the experi-

ments (A) and (B); therefore, these quantities are added to both terms of the difference $(N' - N)$ without altering their values. The volume of CO_2, ascertained during the calorimetric test, is obtained by the difference between the quantity of CO_2 in (B) and the quantity of CO_2 determined in (A).

This is the technique which we are adopting in our experiments and, hereafter, unless advised to the contrary, such results as we may submit will be obtained by same.

As will be seen in this paper, the problem solved consisted in originating a technique for determining the amount of gases of the blood, and with this we obtained the results shown in the following table.

Table showing the result of experiments made on liver emulsion by following the new technique

ORDER NO.	CO_2	O_2	$\frac{CO_2}{O_2}$	HEAT GENERATED (Q)	THERMICAL COEFFICIENT OF O_2 — $\frac{Q}{O_2}$	THERMICAL COEFFICIENT OF CO_2 — $\frac{Q}{CO_2}$
Experiment I.....	382.5	862.5	0.44	2473.0	2.86	6.46
Experiment II.....	408.0	899.6	0.45	3520.0	3.91	8.62
Experiment III.....	380.7	688.5	0.55	2686.7	3.90	7.05
Experiment IV.....	318.0	756.2	0.42	2534.7	3.35	7.97
Average.....	372.3	801.7	0.46	2803.6	3.50	7.52

Comparing this table with the previous one, it will be observed that the amounts of CO_2 obtained by the method derived from that of Batelli and Stern[5] are somewhat higher than they should really be. Where, however, a truly great difference may be observed—so great, indeed, that, in our opinion, it annuls the value of those experiments—is in the amounts of O_2 shown. As a consequence of this, the value of the respiratory quotient, which is very low and constant in signification in our last table, is, on the contrary, very high and of entirely different (and, one may say, opposite) signification in the preceding table.

These differences in the value of the gases measured naturally cause considerable variation in the thermical coefficients of CO_2 and O_2. It should be noted, however, that the calorimetric measurements, in any of this series of experiments, are valid and independent of the

[5] Only in regard to the manner of treating the tissues, as we measured the gases by means of an eudiometric glass, and not, as did those authors, by processes which are not reliable.

amounts of respiratory exchanges; if, in the first table, they are somewhat smaller this should be attributed exclusively to the influence of the environment in which the tissues breathe (namely, the suspension of red globules in Locke's fluid), as we have also remarked that the exchanges of gas are more active in defibrinated blood than in that environment. If we examine only the last table, the figures whereof are reliable, a few interesting facts will be observed: In the first place, the value of the thermical coefficients of O_2 or of CO_2, or the average of the values thereof, differ considerably from those that are used in the respiratory calorimetry; therefore, had we calculated the heat produced by the liver, multiplying by such values the O_2 consumed, the result obtained would have been widely different from the real figures. Then, the thermical coefficients obtained for O_2 are always very much lower, whether we consider each by itself, or take the average of the thermical coefficients of the alimentary substances (protein, fat or glycose). The thermical coefficients of CO_2 are, on the contrary, excessively high for these same alimentary substances. This coincides with (and is, in part, explained by) the extremely low value of the respiratory quotients, the average of which is but 0.46.

Comparing these different values of the respiratory quotients and those of the thermical coefficients of O_2 and of CO_2, we may, by a perfectly legitimate induction, infer, to a certain extent, the nature of given chemical reactions which are taking place in the liver. Thus we can state that in all our experiments, as shown in the aforesaid table, a pronounced transformation of fat into glycose or glycogen occurred in the liver. It is this transformation which explains, to a certain extent, the figures obtained. The liver works "in vitro," separated from the organism, and, therefore, from the pancreas, like a diabetic liver. The determination of the heat by the different organs "in vitro," and the measurement of the amounts of respiratory exchanges, by the aforesaid process, can therefore serve (and should serve) as a good method for the study of the nature of the metabolism of each organ, in its intensity and quality, and, consequently, should also serve for the study of the reciprocal action of the organs on each other.

In our next paper we shall go into the conditions which are necessary, in order to determine the nature of the combustions which occur in an organ, taking as a starting point the heat and the CO_2 and O_2 respectively produced and absorbed by it. At the same time we shall apply these principles to the study of the action of the pancreas on the liver.

CONCLUSION

1. The method employed by Battelli and Stern for determining the amount of respiratory exchanges of the emulsion of tissues, is fallacious, as the results obtained are erroneous.

2. A new technique is originated for the rigid measurement of respiratory exchanges of tissues suspended in defibrinated blood, which procedure is also applicable to the more simple process of determining the amount of gases in the blood.

3. We have determined the amount of heat, and the value of the respiratory exchanges of the liver separated from the organism.

4. We have demonstrated experimentally that with the data at present accepted in physiology, it is not possible to determine the amount of heat produced by the liver, by means of respiratory calorimetry.

BIBLIOGRAPHY

(1) DE ALMEIDA: Researches on the exchange of energy in live animal tissues. Micro-calorimetry applied to animal tissues. This Journal, 1915, xxvii, 505.

(2) BATTELLI ET STERN: Recherches sur la respiration principale et la respiration accessoire des tissus animaux. Compt. rendus de la Societe de Biologie, 1909, lxvi, 372.

(3) BATTELLI ET STERN: See a series of papers published in the Journ. de Physiol. et Path. Gen., 1907.

(4) ABDERHALDEN: Handb. der Biochem. Arbeitsmethoden, 1910, iii, 683.

COMPARISON OF THE AMOUNT OF CATALASE IN THE MUSCLES OF LARGE AND OF SMALL ANIMALS

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The surfaces of two spherical bodies are to each other as the two-thirds power of their masses, hence the amount of surface per unit mass of the small body is greater than that of the large one. On account of this greater exposed surface the small body loses heat more rapidly than the large one. Similarly a small animal loses heat more rapidly than a large one, this greater loss of heat being made good by a greater production. Bergmann (1) was among the first to show that small animals require relatively more food and produce relatively more heat than large animals of the same species, that is the oxidative processes in small animals are more intense than in large animals. These facts have been expressed by Rubner (2) in the so-called Surface Area Law, according to which metabolism is proportional to the surface area or for the same amount of surface area there will be the same production of heat. Burge (3) showed that when oxidation is increased in an animal by increasing the amount of exercise, or decreased by decreasing the amount of exercise, the catalase content of the muscles of the animal is correspondingly increased or decreased. Hence, the conclusion was drawn that there is a close relationship between the amount of catalase in a muscle and the intensity of the intra-muscular oxidative processes. If such a relationship exists there should be more catalase in the muscles of small animals than in the corresponding muscles of large animals. The following experiments were carried out to determine if there is more catalase in the heart muscle of small animals than in the heart muscle of large animals. The heart was chosen because it was thought that the catalase content of this muscle would be less affected by the mode of life of the animal than any of the other muscles. The animals used in one series were the goose, pigeon and English sparrow; those in another, the dog, rabbit and rat. The geese were raised by a local farmer, the pigeons

and English sparrows were birds accustomed to the range of the town, the dogs were tramp dogs, the rabbits were raised in captivity, and the rats were wild field rats. The catalase content of the hearts of these animals was determined according to the following method. The hearts were removed from the etherized animals and a cannula tied into the aorta with its point toward the heart. The cannula was then attached to a pressure bottle containing 0.9 per cent sodium chloride at 38°C. Thus the liquid was passed through the coronary vessels while the heart was still beating until the heart was washed free of blood. Each bloodless heart was ground up separately in a hashing machine and 1 gram of the material was introduced into a bottle

TABLE 1

After heart, under goose, pigeon and English sparrow, are given the number of cubic centimeters of oxygen liberated in ten minutes from 45 cc. of hydrogen peroxide by the catalase in 1 gram of the heart of the respective animal

ANIMAL	1	2	3	4	5	AVERAGE
Goose						
Heart	44	42	50	44	52	46
Weight of goose in grams	6000	6500	6700	7000	6120	6464
Pigeon						
Heart	110	100	98	105	109	104
Weight of pigeon in grams	324	315	330	340	328	327
English Sparrow						
Heart	133	111	122	129	113	121
Weight of sparrow in grams	26	30	24	32	28	28

containing 45 cc. of hydrogen peroxide and as the oxygen gas was liberated it was conducted to a burette previously filled with water. A full description of the method is given in a previous publication (3). After the oxygen gas, collected as described, had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in one gram of the hashed heart. The results of the determinations are given in tables 1 and 2.

It may be seen in table 1 that the average amount of oxygen liberated by one gram of the goose heart was 46 cc.; by 1 gram of the pigeon heart 104 cc.; and by 1 gram of the English sparrow heart 121 cc. The average weight of the geese was 6464 grams; that of the pigeons, 327 grams; and that of the English sparrows, 28 grams; hence the amount of

catalase is greatest per gram of heart in the smallest birds, less in amount in birds of larger size and least in the largest birds.

It may be seen in table 2 that the average amount of oxygen liberated from hydrogen peroxide by 1 gram of the dog heart was 63 cc.; by the rabbit heart, 107 cc. and by the rat heart, 137 cc. The average weight of the dogs was 19,300 grams; that of the rabbits, 2540 grams; and that of the rats, 182 grams; hence the amount of catalase is greatest per gram of heart in the smallest mammal, less in amount in the larger mammal and least in the largest mammal.

Determinations (4) have been made of the catalase content of a great many different kinds of insects. It was found that a single firefly, weight 11 mgm. ground up in sand liberated about 50 cc. of

TABLE 2

After heart, under dog, rabbit and rat, are given the number of cubic centimeters of oxygen liberated in ten minutes from 45 cc. of hydrogen peroxide by the catalase in 1 gram of the heart of the respective animals

ANIMAL	1	2	3	4	5	AVERAGE
Dog						
Heart	50	75	73	53	68	63
Weight of dog in grams	25,000	12,000	16,000	27,500	16,000	19,300
Rabbit						
Heart	76	74	82	91	70	79
Weight of rabbit in grams	2,300	2,600	2,000	2,272	2,640	2,332
Rat						
Heart	133	145	140	115	150	137
Weight of rat in grams	200	210	250	150	100	182

oxygen from 45 cc. of hydrogen peroxide in ten minutes; that is, 1 gram of firefly would liberate about 5000 cc. of oxygen in ten minutes. Other insects such as the honey-bee, bumble-bee, moth, etc., were found to be very rich in catalase, but not so much so as the firefly. It will be noted that 1 gram of firefly contains about one hundred times as much catalase as 1 gram of dog heart.

Several observers (5) have pointed out instances in which the Surface Area Law does not hold. Lusk (6) showed that the metabolism of a dog kept in the laboratory decreased very considerably (19 per cent) from what it was when the dog was kept in the country, although the weight of the dog, and hence the surface area, remained constant. Benedict (7) showed that the metabolism of a man during a thirty-one-day fast decreased considerably more than what could be accounted

for by the decreased surface area during the fast. He also observed that the metabolism rose during the last few days of the fast although the surface area of the subject was still decreasing. The failure of the law in some instances has been attributed to the inaccuracy in methods for determining the surface area, nature of the integument, variations in amount of body surface exposed, etc., but with more exact methods for determining the surface area it is still found that the law does not hold under many normal as well as pathological conditions, hence Benedict (7) concludes that the loss of heat from the body surface can not be the determining factor and that of the numerous factors affecting metabolism, the internal stimulus to metabolic activity is one of the greatest.

Burge (3) showed that when pigeons accustomed to an active life were confined, the catalase content of the pectoral muscles decreased by 25 to 30 per cent although the weight of the animals remained constant, and that when the animals were given their accustomed amount of exercise the catalase content increased to the normal amount. It is not improbable that the decrease of 19 per cent in the metabolism of Lusk's dog leading an inactive life was due to a decrease in the catalase content of the tissues of the animal. In some unpublished results from this laboratory it was found that the catalase content of the muscles of rats during the first few days of fasting was increased above the normal particularly when the animal was given a small amount of protein. This observation carries the suggestion that the specific dynamic action of proteins may be explained by an increase in catalase, a suggestion which we hope to follow out experimentally.

SUMMARY

Evidence is presented to show that the content of heart-muscle in catalase, per unit of weight, bears an inverse relationship to the size of the animal, or a direct relationship to the intensity of the oxidation processes.

BIBLIOGRAPHY

(1) BERGMANN: Ueber die Verhältnisse der Wärmeokonomie der Thiere zu ihrer Grösse, Göttingen, 1848.
(2) RUBNER: Zeitschr. f. Biol., 1883; xix, 535; und Gesetze der Energieverbrauchs, 1902.
(3) BURGE: This Journal, 1916, xli, 153.
(4) BURGE: Journ. of the Franklin Institute, 1916, clxxxii, 263.

(5) BENEDICT, EMMES, ROTH AND SMITH: Journ. Biol. Chem. 1914, xviii, 139; BENEDICT AND ROTH: Ibid, 1915, xx, 231; BENEDICT AND SMITH: Ibid, 1915, xx, 243; BENDEDICT AND EMMES: Ibid., 1915, xx, 253; BENEDICT: Ibid., 1915, xx, 263.
(6) LUSK: Journ. Biol. Chem., 1915, xx, 565.
(7) BENEDICT: This Journal, 1916, xli, 292.

THE SIGNIFICANCE OF PROTHROMBIN AND OF FREE AND COMBINED THROMBIN IN BLOOD-SERUM

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The fresh serum expressed from the blood clot when added to oxalated plasma or to fibrinogen soon produces coagulation. This thrombic power of serum decreases rapidly, especially at body temperatures, but may be reestablished by various procedures, such as addition of tissue extract or by a transient increase in alkalinity. The nature of these inactive stages of thrombin has been the subject of a number of investigations, the result of which has been considerable difference of opinion in regard to the interpretation of the different procedures of activation. It will be shown as a result of the following experiments that they are capable of a simple explanation which does not involve the assumption of any substance other than those quite generally recognized as coagulation factors.

It was the opinion of Alexander Schmidt (1) that "prothrombin" is only partially activated[2] in the process of coagulation and appears in serum together with thrombin. He believed that prothrombin remains in serum much longer than thrombin and may be activated by the addition of zymoplastic (thromboplastic) substance or by a transient increase of alkalinity, or by both, the alkalinity favoring the action of the zymoplastic substance.

A distinct advance was made by Morawitz (2). He found that there is more than one proferment. He did not believe that the proferment activated by calcium, described by Pekelharing (3) and which we now know as prothrombin, could exist in serum, as blood contains sufficient

[1] This work was started in the physiological laboratory of Johns Hopkins University and most of the experiments were performed at that place.

[2] Schmidt used the word 'Spaltung.' The translation activation is merely the commoner terminology and is not intended to designate the true nature of the process.

calcium to activate all the prothrombin, and addition of calcium to serum does not increase its thrombic power. However, he described a proferment distinct from that of Pekelharing which is activated by a change of the H^+ ion concentration of the serum. This he believed was identical with the "prothrombin" of Schmidt. To distinguish the two proferments he called the one activated by calcium α proferment, and the one activated by a change of the H^+ ion concentration β proferment. β proferment does not exist in the circulating blood or oxalated plasma but exists in serum, whether fresh or old.

In the same year Fuld (4) described the substance in serum which he called metazyme, which is activated by alkali or acid and which he regarded as a more stable phase of thrombin. This substance is to be regarded as identical with the β prothrombin of Morawitz. The latter did not agree that this substance is a more stable phase of thrombin but offered no other explanation. As it seemed most probable that the substance was a modification of thrombin, he suggested the term "metathrombin." For the sake of convenience this term will be used to denote the substance or condition which admits of the development of thrombin when serum is treated with alkali. In a later paper Morawitz (5) revised his view that α prothrombin does not exist in serum as he found it to contain a thrombogen[3] activated by tissue extract in the presence of calcium. That this thrombogen is not identical with metathrombin was evident from the fact that a serum may contain the latter when the former has disappeared. He further presented two other facts which have been confirmed in this investigaiton; serum may be activated by alkali and acid in the absence of calcium but cannot be activated by tissue extract unless calcium is present.

Pekelharing (6) objected to the assumption of the two ferments by Morawitz. It is his belief that serum contains thrombin but no proferment. When serum stands exposed to the air substances arise which oppose the coagulation of fibrinogen and these substances are rendered inactive by acid and alkali. These inhibiting substances are stated not to act on thrombin (7). However, Landsberg (8) came to the conclusion that thrombin is inactivated by adsorption on to the serum proteins. Such an adsorption compound corresponds to the metathrom-

[3] Morawitz differentiates this thrombogen from α prothrombin. The α prothrombin was originally defined as a substance activated by calcium. Later when he introduced his theory of a thrombokinase he defined α prothrombin as kinase plus thrombogen without calcium.

bin of Morawitz. The adsorption takes place in the same manner that trypsin, emulsin and rennin are taken up and inactivated by charcoal, casein or serum albumen.

Weymouth (9) studied the problem of the activation of serum and came to the conclusion that the substance described as metathrombin is a combination of thrombin with the antithrombin of the serum. The contention of Mellanby (10) and of Collingwood and MacMahon (11) that such a combination is not broken up by the alkali-acid activation is readily disproved. These authors believe that what really happens is a liberation of thrombokinase from the serum proteins on to which it has been adsorbed. If this were the case then the phenomenon would not take place if oxalated serum were activated and tested on oxalated plasma.

METHODS

Only a brief note as to methods and materials used is necessary. Thrombin was prepared by the method described by Howell (12). For the thromboplastic substance occurring in tissue extract cephalin (13) was used. Fibrinogen was prepared by precipitation from oxalated plasma by half saturating with sodium chloride, the precipitate formed being aggregated by the centrifuge, washed with a half saturated solution of sodium chloride and dissolved in a 2 per cent solution of sodium chloride. This procedure was repeated three times and only such preparations were used as did not give a clot in twenty-four hours, when calcified in the presence of cephalin. In some experiments where the action of calcium did not enter oxalated plasma was used. The coagulation time was determined in homoeopathic phials. The coagulation times recorded in this paper, unless otherwise noted, were taken at the time that the phial could be inverted without disturbing the clot.

The method of alkali activation used was practically the same as that used by previous observers. The serum was incubated at 37° for ten minutes with an equal volume of $\frac{N}{10}$ NaOH, then neutralized with $\frac{N}{10}$ HCl using neutral red as indicator. In the later experiments it was found that room temperature was equally satisfactory.

Antithrombin (14) was determined by incubating serum (heated to 60° for ten minutes to remove thrombin and prothrombin) with thrombin for a definite time in the incubator at 37°, then determining the ability of the mixture to coagulate fibrinogen.

EXPERIMENTS

There are two possibilities as to the origin of thrombin in activated serum. It may arise from some alteration of thrombin itself, either by a change in structure of the thrombin on standing or its modification in some way by substances present or developing in serum, or it may arise from prothrombin such as exists in plasma. The former possibility will be first considered.

It has been the general experience of workers with blood that aqueous solutions of isolated thrombin on standing do not undergo inactivation. This fact makes it probable that the inactivation of thrombin in serum is not due to a change in the structure of thrombin. We must turn then to the other alternative, that thrombin is inactivated in serum through the intervention of some substance contained in the serum.

Landsberg considered that the thrombin is adsorbed on to the serum proteins just as rennin may be adsorbed by such a substance as casein. Morawitz (2) tried the action of eggwhite, milk, dextrin and commercial egg albumen on coagulation of fibrinogen by serum and found that none of them caused an appreciable delay. Rettger (15) postulated that if thrombin unites with a protein there must be a marked specificity in this respect as serum soon inactivates thrombin, while but little change takes place when thrombin stands in contact with egg albumen. That there is a specific antithrombin in the blood has gained considerable support from Howell and his coworkers, and Weymouth decided that the metathrombin of Morawitz is a combination of such a substance and thrombin. Therefore in this investigation for comparison with serum a series of determinations of the antithrombin power of various substances was made. Serum was heated to 60° to remove its thrombin and then amounts of one drop were incubated for fifteen minutes at 37° with 2, 3 and 4 drops of thrombin solution. Under exactly duplicate conditions the following substances were incubated with like amounts of the same thrombin solution: (1) Fresh whole milk; (2) fresh egg albumen; (3) peptone ("Witte") 6 per cent solution; (4) potato starch (a thick paste); (5) acacia, 6 per cent solution; (6) gelatine, 6 per cent solution; (7) animal charcoal, 6 per cent suspension; (8) kaolin, 6 per cent suspension.

The thrombin used was of such a strength that two drops clotted ten drops of oxalated plasma in 1 + minutes. When incubated with the above mentioned substances for 15 minutes all the preparations

clotted in between 1 and 2 minutes, on the other hand the thrombin incubated with the serum antithrombin was so much inactivated that determination of a definite end point was impossible as is seen in the following data.

ANTITHROMBIN	INCUBATED 15 MIN. WITH THROMBIN	COAGULATION TIME
1 drop	2 drops	Pellicle 2 hours; did not get solid
1 drop	3 drops	Pellicle 75 minutes; gel 4 hours solid 8–20 hours
1 drop	4 drops	Weak gel 20 minutes, solid 8–20 hours

The above experiment shows that the inactivation of thrombin by other substances than serum is negligible when compared with that of serum, in fact it is so small that it could not be measured by the method used. This fact allows of but one conclusion, that the action between serum and thrombin is of a specific nature.

We have seen that thrombin is inactivated by serum, it remains to be shown that a union is formed which answers to the specifications of metathrombin.

It was argued by Morawitz that metathrombin could not be such a combination, as it occurs in old serum from which antithrombin has disappeared. As we now have improved methods (14) of testing for antithrombin this work was repeated. Serum obtained on September 29 was allowed to stand in a stoppered bottle on the laboratory shelf and was tested from time to time for metathrombin and antithrombin. These were found to occur together. November 27 was the last day on which any thrombin could be developed by alkali activation. On this day the antithrombin test was as follows.

THROMBIN	INCUBATED FOR 15 MIN. 37°C WITH	CLOTTED 10 DROPS OF OXALATED PLASMA IN
4 drops	Water 2 drops	8 minutes
4 drops	Serum 2 drops	Jelly clot over night

After this no thrombin could be developed by alkali activation and antithrombin tests showed no appreciable amount of this substance.

A study of the susceptibility of the serum constituents to heat furnished additional evidence of a thrombin-antithrombin combination. Thrombin is destroyed at a lower temperature than antithrombin. It is to be expected therefore that the susceptibility of the combined-thrombin would approximate that of antithrombin in a manner similar

to the protection afforded to heat by a substrate towards an enzyme, as peptone to trypsin, Biernacki (16). It is stated by Morawitz (17) and confirmed by Weymouth (9) that thrombin and metathrombin have the same thermolability. On the other hand Rettger (15) found that if a serum be heated for a few minutes at 65° it is inactive when added to a fibrinogen solution but becomes active on treating with alkali and acid.

At a proper selection of temperature and duration of heating it was found that there is a definite difference in susceptibility of thrombin, prothrombin and metathrombin. Serum and plasma were heated side by side in thin walled test tubes in a water bath to the temperature in-

TABLE 1

SERUM AMOUNT HEATED	COAGULATION TIME		ANTITHROMBIN DETERMINATION		
	Serum 5 drops + fibrinogen 10 drops	Serum 5 drops, activated with alkali + fibrinogen 10 drops	Thrombin 2 drops	Thrombin 3 drops	Thrombin 4 drops
unheated	90 min.	10 min.	flocculent precipitate 60 min.	gel 45 min.	10 min.
60.0°	No clot	20 min.	flocculent precipitate 60 min.	gel 45 min.	10 min.
62.5°	No clot	60 min. good gel 35 min.	Pellicle 60 min.	10 min.	3 min.
65.0°	No clot	gel 60 min. solid over night	gel 3 min.	1 min.	1 min.

dicated and held there accurately for five minutes. To remove the fibrinogen from the plasma it had been previously heated to 55° and the precipitate removed by the centrifuge. In both plasma and in serum heated to 60° or over no substance remained which yields thrombin on the addition of calcium in the presence of thromboplastic substance. The fate of thrombin, metathrombin, and antithrombin is shown in the following table. In the figures given for antithrombin one drop of serum was heated with the indicated amount of thrombin for 15 minutes at 37°. The thrombin was of such a strength that two drops clotted ten drops of fibrinogen solid in 1 minute.

It is to be seen that progressive heating to 65° injures metathrombin, but 65° for 5 minutes does not destroy it. At the same time the

antithrombin is weakened and but little remains at 65°. Above this temperature both are destroyed. On the other hand all sera heated to 60° or more are inactive, as regards free thrombin, showing a clean cut separation by heat of thrombin and metathrombin, the combined-thrombin being destroyed, not at its own critical temperature in serum but at that of antithrombin. This is good evidence that thrombin is in combination with the antithrombin and controverts the view of Pekelharing that the phenomenon of alkali activation is due to the removal of substances developing in serum which prevent the action of thrombin on fibrinogen but which do not combine the thrombin.

If the line of reasoning followed in this investigation holds, then the effect of alkali on serum is to release the thrombin from the antithrombin, therefore the effect of alkali activation on the antithrombin of serum was studied. Serum was activated and its thrombic and anti-

TABLE 2

	CLOTTED FIBRINOGEN 10 DROPS	AFTER HEATING AT 60° FOR 10 MIN. AND INCUBATING FOR 10 MIN. WITH THROMBIN 2 DROPS—CLOTTED FIBRINOGEN 10 DROPS
Serum 4 drops (unactivated)	55 min.	No clot 21 hours.
Serum 4 drops (activated)	10 min.	75 min.
Serum 4 drops (reactivated)	60 min.	5 min.
Thrombin 2 drops	5 min.	

thrombic power determined. It was then heated to 60° for 10 minutes to remove the free thrombin, after which it was reactivated and a determination made of the thrombin and antithrombin, for comparison with the unactivated serum and the serum activated only once. Care was taken in each case that the determination was made on an amount equivalent to four drops of the original serum. The results are here tabulated.

It becomes apparent from these experiments that in the process of alkali activation, simultaneous with the increase of thrombic power of the serum there is a decrease in its ability to inactivate thrombin. This ability becomes nil with the second activation, a fact which explains the statement made by Morawitz that serum can not be activated a third time; there is no antithrombin remaining in condition to combine thrombin.

The most direct proof of the nature of metathrombin is to be ob-

tained by addition of thrombin to antithrombin. Experiments of this kind (9) have been tried, but it is obvious that an error is introduced when serum is used. In order to heat the serum sufficiently to remove the metathrombin already present would require a temperature that would destroy the antithrombin. Therefore oxalated plasma which had been heated to 60° to remove the prothrombin was used for antithrombin as it has been repeatedly demonstrated that plasma does not contain metathrombin. It was found that small amounts of plasma could inactivate very large amounts of thrombin in a few hours, therefore the plasma was added directly to the watch crystals containing the dried thrombin. The presence of thrombin after alkali activation is not sufficient proof of activation, the free thrombin already present is injured but by no means destroyed in the process. The criterion of a thrombin-antithrombin combination was a plasma which had but slight thrombic activity but which developed thrombin after treatment with alkali and acid. Such a preparation can be obtained with great regularity if the plasma is incubated for the proper time with sufficient thrombin.

The data of two of the most successful experiments are presented as follows:

Twenty drops of plasma were added to a watch crystal in which 5 cc. of a very active thrombin solution had been dried. The mixture was allowed to stand for 24 hours at 30 to 35°, at the end of which time it reacted as follows:

A

Thrombin-plasma mixture, 6 drops + oxalated plasma 10 drops, no clot in 24 hours.

Thrombin-plasma mixture, 6 drops (activated) + oxalated plasma 10 drops, clot in 6 minutes.

Cephalin had no effect on this combination.

In an experiment similar to the above in which different preparations of the constituents were used the following results were obtained:

B

Thrombin-plasma mixture, 5 drops + fibrinogen 10 drops, no clot in 10 hours.

Thrombin-plasma mixture, 5 drops (activated) + fibrinogen 10 drops, clot in 1 hour, gel in 30 minutes.

From the foregoing experiments we may conclude that in serum there exists a combination of thrombin and antithrombin which has

the thermolability of the latter, not of the former, which does not yield thrombin on the addition of cephalin but is disrupted by alkali with a liberation of thrombin attended by such an alteration of antithrombin that it becomes inactive.

In addition to the combined-thrombin existing in serum there is also evidence of the presence of prothrombin as was believed by Schmidt and Morawitz. If a small amount of cephalin is added to fresh serum it becomes very active. If the serum is first oxalated no activation occurs. That this is not due to the excess of the oxalate can be shown as no activation occurs after removal of the excess of oxalate by dialysis. That oxalate produces no injury to the prothrombin follows from the observation that normal activation of such a serum by cephalin occurs if it is recalcified. The only substance known in the coagulation system that yields thrombin in the presence of calcium and tissue extract is prothrombin. The thrombin-antithrombin union when once formed has been shown not to be affected by thromboplastic substance as serum heated to 60° for 10 minutes does not develop thrombin on the addition of cephalin and the latter does not split the union between plasma heated to 60° and thrombin.

The time at which the thrombin is combined by the antithrombin is of interest. No combined-thrombin exists in oxalated plasma but it is present in apparently full amount in the fresh serum. The following experiment was devised to determine the time of its origin and the relationship of the amount of tissue extract to the amount of the different stages of thrombin in serum. In order to get a plasma low in tissue-extract content a cat was bled from the carotid through a greased cannula into oxalate. This was centrifuged at once at high speed for over an hour to obtain a very clear plasma. Such a plasma was heated to about 55°C., at which point the fibrinogen is coagulated but the other coagulation factors are undisturbed. The coagulum was removed by means of the centrifuge. If a plasma so prepared is calcified the process of thrombin formation is initiated without the removal of any of the thrombin by fibrinogen, furthermore it is not necessary to wait till after clot formation to study the serum. The plasma was calcified according to an optimum previously determined on the unheated oxalated plasma.

Two series were run simultaneously: Preparation I was low in tissue-extract content (i.e., contained only the small amount unavoidably present in the oxalated plasma); Preparation II was prepared with an excess of cephalin. Thrombin determinations were made on these

after oxalating to prevent further conversion of prothrombin. For prothrombin determinations, to Preparation I was added cephalin. Preparation II already having an excess of cephalin was untreated. The coagulation times observed in the prothrombin determinations therefore represent the effect of the thrombin already present plus the effect of that formed from prothrombin after preparation of the coagulating mixture. Combined-thrombin was determined by the alkali activation of a portion that had been heated to 60° for 5 minutes to remove the thrombin and prothrombin present. The slight injury unfortunately occurring in this amount of heating does not seriously interfere with the results. It necessitates, however, that the heating of each specimen should be exactly the same. It is obvious, in experiments of this kind where tissue extract and calcium are both present, that the tests, to be of value, had to be made on a fibrinogen preparation absolutely free from prothrombin, that is without a trace of clotting after a 24-hour contact with calcium and cephalin.

Determinations were made at periods varying from 1 minute to 24 hours after calcifying. The results obtained are plotted in figures 1 and 2.

As the observations lasted 24 hours and as the maximum changes occurred in the first few minutes it was necessary to plot the points on a system of logarithmic coördinates to get the results within the curve. The axis of abscissae represents the time in hours after calcification of the plasma, the axis of ordinates represents the amount of thrombin as indicated by the reciprocal of the coagulation time expressed in hours. It should be borne in mind that the coagulation time is an index to the amount of thrombin only up to a certain maximum amount above which the coagulation time can not be shortened by an increase of thrombin present (15). Therefore in the prothrombin determinations in Preparation I there was during the whole 24 hours at least sufficient prothrombin to give coagulation in the minimum time, one minute or less, except in the last test where the coagulation time was 2 minutes. There must have been a diminution of the prothrombin as thrombin was formed but this never reached a point below the maximum that could be determined. In contrast with this in Preparation II where there was an excess of tissue extract, the prothrombin was converted to thrombin at the very start of the experiment. The prothrombin content of the serum must very soon fall to zero. It is seen that the prothrombin curve follows the thrombin curve very closely. At most only the difference between the two curves can rep-

resent prothrombin and this difference must be still further discounted, as in the preparation on which the thrombin was determined a precipitate of calcium oxalate had been formed which would carry down some of the thrombin.

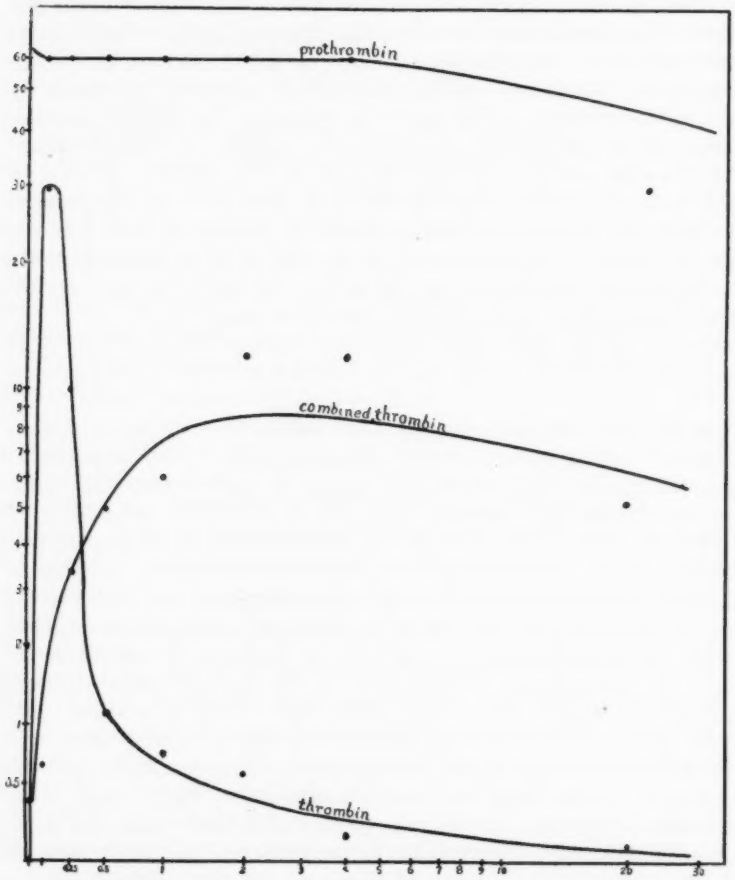

Fig. 1. The development of thrombin and combined-thrombin in a serum formed in the presence of a minimum of cephalin. Logarithmic coordinates. Abscissae: time in hours after calcification of plasma. Ordinates: amount of thrombin as indicated by the reciprocal of the coagulation time expressed in hours.

The rapidity of conversion of prothrombin explains the great variability of sera in relation to tissue extract. In the early part of the work the fact that cephalin would not increase the thrombic power of

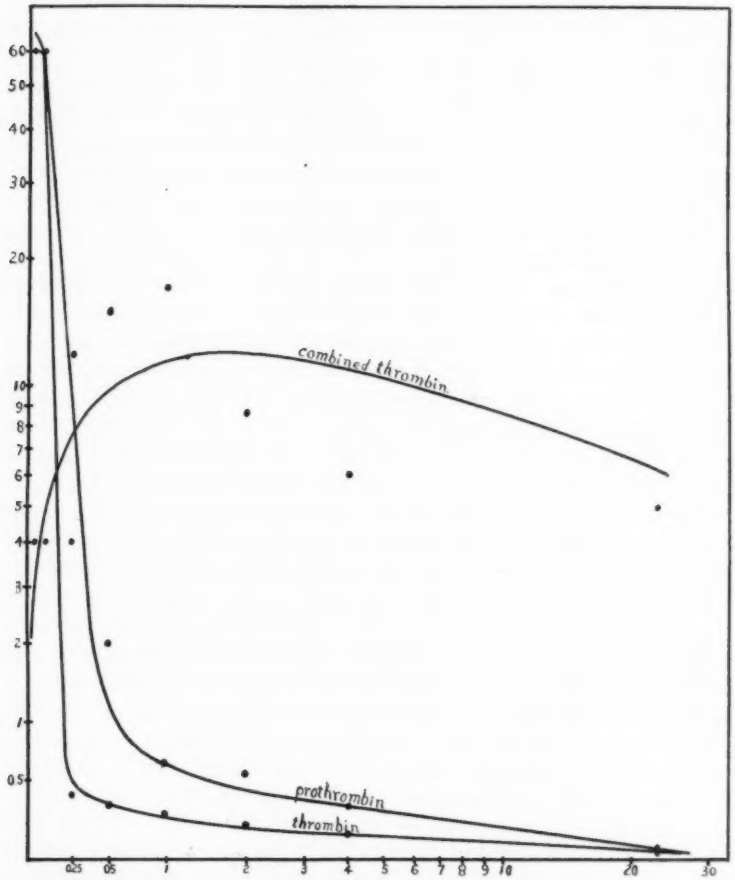

Fig. 2. The development of thrombin and combined-thrombin in a serum formed in the presence of an excess of cephalin.

many sera was a perplexing phenomenon especially as it was not known that cephalin would not liberate combined-thrombin. As the activation of a serum by tissue extract depends upon its prothrombin con-

tent, and this in turn depends upon the amount of tissue extract already present, it can be seen that conversion of prothrombin is so complete when enough of the latter is present during clotting that no more remains in the serum to be liberated by thromboplastic substance.

The thrombin curves in the two cases show two points in common. There is an early rapid rise in amount followed by a rapid fall. The rise is best seen in Preparation I, no thrombin was demonstrable in the one minute sample, but it reached its maximum after 5 minutes. In Preparation II the rise was so rapid that in the one-minute specimen coagulation was in the minimum time. In both cases the disappearance of the thrombin was strikingly rapid. This rapid disappearance of thrombin which is undoubtedly also taking place even while the total amount is rising is attended by an increase in the amount of combined-thrombin comparably rapid.[4] This explains the observation of the older observers that metathrombin is present in maximum amounts in fresh serum. By the time the serum could be obtained from the clot sufficient thrombin had combined to give the maximum reaction. There is no evidence whatever in the curves that thrombin is constantly being combined; the amount of thrombin that the amount of tissue extract is capable of producing is formed rapidly, then quickly disappears so that the maximum of the combined-thrombin curve is reached very early, after which the amount as far as it can be determined remains nearly stationary, at least there is no further rise. The slight fall in the curves is to be explained in part by the deterioration of the fibrinogen which takes place during the period of the experiment. The decrease in combined-thrombin in serum must be very gradual as it is still present in large amounts after several weeks. The apparent variation in the points on the curve is due to the method of plotting. The maximum variation in the coagulation times observed after the curve reaches its maximum, is only 8 minutes and this is greatly influenced by slight variations in the H^+ ion concentration of the end point after neutralization.

[4] The slight injury caused by the heating to 60° prevents the rise of the combined-thrombin curve to the height it would attain if determinations were made on unheated preparations. On the other hand a curve determined under the latter conditions would be very inaccurate in parts of the curve showing large amounts of free thrombin.

DISCUSSION

The foregoing facts have a direct bearing on the procedure of the coagulation process. It is a well known fact that large amounts of thrombin can be injected into the circulating blood without the occurrence of thrombosis. In figure 1 is written the story of a comparable situation. The small amount of thromboplastic substance present in the plasma was capable of forming rapidly a definite amount of thrombin, but this thrombin disappeared about equally as rapidly, the rise of the combined-thrombin curve showing the manner of its removal. As thrombin can not be liberated from the blood antithrombin by any agency known to be present in normal blood the small amounts of thrombin that may arise as a result of thromboplastic substance becoming available are permanently removed from the possibility of activity. In the blood stream the removal would be even more rapid. The experiments were performed at room temperature whereas at body temperature the activity of antithrombin is very much greater, Howell (18). In normal coagulation the thrombin when formed has two possible fates. It may be combined by antithrombin or by fibrinogen. The division coefficient between these two would be a valuable bit of information. It is to be expected that the presence of the fibrinogen would greatly modify the course of the combination of thrombin by antithrombin. We may cite for analogy the relatively greater inhibition of rennin by serum-albumen, Hedin (19), when the rennin has been in contact with the serum-albumen before the addition of the milk. It would seem that such a combination of thrombin by antithrombin would be very detrimental to the process of coagulation but during conditions allowing clotting the coefficient of division would be modified in favor of the fibrinogen combination by the decrease of temperature and by the presence of thromboplastic substance. There is an undoubted delay in the thrombin-antithrombin union in the presence of cephalin as is shown very clearly in Howell's experiments (13). This delay is not sufficient, however, to prevent a rapid development of combined-thrombin when a great deal of thrombin is present (vid. fig. 2), or to maintain thrombin free in the serum for any length of time.

The properties of the thrombin-antithrombin combination have a relationship to the nature and condition of prothrombin. The resistance of the combination to cephalin precludes the possibility that prothrombin is a thrombin held in an inactive form in the circulatory

plasma, at least as an antithrombin combination. According to the theory of coagulation formulated by Howell, prothrombin is protected from calcium activation by antithrombin. Such a prothrombin-antithrombin complex would seem to be different in nature from the thrombin-antithrombin combination as it is broken up by thromboplastic substance and the prothrombin retains its own critical temperature, not being protected by antithrombin.

The facts known at present concerning the nature of the combination in which thrombin is held does not allow of its definite classification. If an adsorption it would be one in which the substance adsorbed is fixed in such a way that the process is not reversible. Hedin (20) has described such a process for the inactivation of enzymes. The inactivation of trypsin or rennin by serum albumen or egg white differs in many ways from ordinary adsorption. The enzyme may be completely removed if sufficient of the inactivating agent is present. The amount of enzyme taken up increases with the temperature and it is independent of the amount of water present, the adsorption compound is therefore not broken down by the addition of water. The fact that a combination of the enzyme with charcoal occurs which has the same properties has led Hedin to class the process with the adsorptions. If the serum albumen or egg white is treated with acid, then neutralized, it loses its power to combine an enzyme. The analogy of the Hedin adsorption with thrombin inactivation led Landsberg to assume that thrombin is adsorbed by serum albumen. In many ways the analogy holds. However, as has been pointed out, the inactivation of thrombin by serum is all out of proportion to its inactivation by charcoal or eggwhite and one would be forced to assume that there is a marked specificity in the reaction whether this be due to a favorable chemical configuration of the surface of the antithrombic phase or a more rapid or complete fixation of the substance adsorbed. The Hedin adsorption is partially reversible. When the substrate of the enzyme is brought into contact with the adsorbed enzyme a part of the substrate is adsorbed and forces out a portion of the enzyme, which acts upon the unadsorbed substrate. In the case of the thrombin-antithrombin combination, fibrinogen does not produce this effect as a serum heated to 60° when added to fibrinogen does not produce a clot although it may be proved by alkali activation to be rich in combined-thrombin. There is at present, in so far as is known to the author, no evidence that thrombin-antithrombin reaction is reversible; processes which break up the combination also injure or destroy the antithrombin.

SUMMARY

1. There is only one kind of thrombin in serum. This may be formed from prothrombin by the action of thromboplastic substance and calcium or may be liberated from a combination with antithrombin by alkali activation according to the method of Schmidt.

2. The inactive stage from which thrombin is liberted by alkali activation is believed to be a thrombin-antithrombin combination for the following reasons:

(a) The inactivation of thrombin by non-specific colloids, charcoal and kaolin is negligible compared with the inactivating action of plasma.

(b) Combined-thrombin and antithrombin disappear at the same time from serum allowed to stand.

(c) Combined-thrombin is destroyed by heat not at the critical temperature of thrombin but at that of antithrombin.

(d) In the process of alkali activation the antithrombin is destroyed or so altered that it loses its ability to combine thrombin.

(e) When thrombin is incubated with antithrombin (i.e. plasma heated to 60°) a combination is found which does not clot fibrinogen until broken up by the alkali activation method.

3. Thrombin is not liberated from combination with antithrombin by the action of thromboplastic substance.

4. The amount of prothrombin in serum varies inversely as the amount of tissue extract present at time of clotting.

5. The amount of thrombin formed in the presence of a given amount of thromboplastic substance is, apparently, definite in amount; it forms rapidly and is combined rapidly, the system then returning to equilibrium. This rapid inactivation is a potent factor in the maintenance of the normal fluidity of the blood. Plasma is very intolerant of free thrombin.

It is a great pleasure to me to thank Dr. Howell for suggesting this problem and for the kindly suggestions received during its solution.

BIBLIOGRAPHY

(1) Schmidt: Zur Blutlehre, 1892, 201–210.
(2) Morawitz: Beitr. zur chem. Physiol. und Path., 1903, iv, 381.
(3) Pekelharing: Deutsch. med. Wochensch., 1892, 1133.
(4) Fuld: Zentralbl. f. Physiol., 1904, xvii, 529.
(5) Morawitz: Deutsch. Arch. f. klin. Med., 1904, lxxix, 1.

(6) PEKELHARING: Biochem. Zeitschr., 1908, xi, 1.
(7) PEKELHARING: Zeitschr. f. physiol. Chemie, 1913, lxxxv, 341.
(8) LANDSBERG: Biochem. Zeitschr., 1913, l, 245.
(9) WEYMOUTH: This Journal, 1913, xxxii, 266.
(10) MELLANBY: Journ. of Physiol., 1908, xxxviii, 28.
(11) COLLINGWOOD AND MACMAHON: Journ. of Physiol., 1912, xlv, 119.
(12) HOWELL: This Journal, 1913, xxxii, 264.
(13) HOWELL: This Journal, 1912, xxxi, 1.
(14) HOWELL: Arch. of Int. Med., 1914, xiii, 76.
(15) RETTGER: This Journal, 1909, xxiv, 406.
(16) BIERNACKI: Zeitschr. f. Biol., 1891, xxviii, 49.
(17) MORAWITZ: Ergebn. d. Physiol., 1904, iv, 367.
(18) HOWELL: This Journal, 1914, xxxvi, 1.
(19) HEDIN: Zeitschr. f. physiol. Chemie, 1909, lx, 102.
(20) HEDIN: Ergebn. d. Physiol., 1910, ix, 433.

THE POSTURAL ACTIVITY OF THE ABDOMINAL MUSCLES OF THE CAT

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Sherrington (1) has recently called attention to the general postural activity of muscle and nerve in animals. In a study of the neuro-muscular respiratory mechanism we had occasion to investigate the rôle of the abdominal muscles in the respiratory movements. In the course of these experiments we were impressed with the great variations in length of the rectus abdominis muscle, and decided to look into the cause of these variations a little more closely.

Our experiments were done on cats under continuous ether anesthesia and without decerebration. Respiratory tracings were taken from the thorax and the abdomen by means of a Crile stethograph (2), connected to a Verdin recording tambour. The rectus abdominis muscle was exposed by dissecting away the lower medial portion of the pectoral muscles of one side and exposing the attachment of the rectus abdominis to the ribs. A thread attached to the upper end of the rectus abdominis, after severing its attachments to the ribs and dissecting it down to the beginning of the transversalis abdominis, was attached to a muscle lever whose writing point was directly beneath that of the recording tambours. The movements of the lever indicated the variations in the length of the muscle, a fall of the lever indicating a relaxation. Some movements of the muscle lever occurred with each respiration, but these ordinarily were not very great. In dyspnoea the movements of the rectus abdominis might increase very greatly. This phase of its activity will, however, be discussed in a separate communication. We wish, at this time, simply to call attention to its active changes in length with changes in the intra-abdominal pressure.

Our first observations were made by distending the stomach with fluid by means of a stomach tube inserted through the esophagus. To prevent regurgitation when the pressure of the fluid became more

than a few centimeters of salt solution we found it necessary to pass a ligature about the esophagus in the neck. We discovered that it was impossible, however, to reach the limits of distension of the abdominal wall by injecting fluid into the stomach. We therefore injected an $\frac{M}{8}$ solution of sodium chloride directly into the peritoneal cavity by means of a burette and a hypodermic needle. The injection of the fluid was usually continued until no more would run in under a pressure of a column of fluid 80 to 100 cm. high.

When the central nervous system and the afferent nerve roots are intact, the introduction of 100 cc. of fluid into the stomach or the peritoneal cavity is attended by a slight transient increase in the abdominal girth, as shown by the tracing of the abdominal respiratory movements, and a marked increase in the length of the rectus abdominis muscle. The introduction of small amounts of fluid, 10 to 25 cc., may be sufficient to bring about a noticeable change in the length of the rectus abdominis and a small increase in the girth of the abdomen. Subsequent injections of fluid in quantities of 100 cc. are, of course, attended by an increase in the abdominal girth and by a lengthening of the rectus abdominis muscle until the limit of distensibility of the muscle is reached. The pressure under which the first injections were made was not more than 5 or 10 cm. of salt solution, and fluid will continue to enter the peritoneal cavity at these low pressures of injection until one approaches the limits of extensibility of the abdominal wall, when the pressure required for injection rather suddenly rises. When the pressure of injection becomes relatively high, practically every trace of diaphragmatic respiration ceases, as shown by the minimal excursion of the writing point of the tambour connected with the abdominal stethograph. The costal respiration increases as the abdominal respiration becomes more and more feeble. We cite below a protocol of one typical experiment, in which the fluid was injected directly into the stomach (A) when the central nervous system was intact and (B) after section of the dorsal roots of certain spinal nerves.

Cat. June 14, 1916

Ether. Tracheotomy. Laminectomy before any records were taken, but the dorsal nerve roots were not divided at first.

Fluid introduced into stomach through a stomach tube. Approximately $\frac{1}{4}$ molecular solution of sodium chloride was used.

12.40 a.m. 100 cc. fluid injected. Left rectus abdominis muscle prepared.
12.44 a.m. 100 cc. fluid injected.
12.45 a.m. 100 cc. fluid injected. See figure 1.

12.51 a.m. 100 cc. fluid injected.
12.54 a.m. 100 cc. fluid injected.
12.56 a.m. 100 cc. fluid injected.
12.58 a.m. 100 cc. fluid injected.
1.00 p.m. 84 cc. fluid injected.

NOTE. The fluid was observed to flow into the stomach during inspiration, and to remain stationary in the burette or to flow in more slowly at other times. The pressure at which the flow into the stomach ceased was 37 cm.

As we desired to test out the reactions of the same animal after section of the dorsal nerve roots, the end of the stomach tube was depressed and the fluid allowed to flow out of the stomach. In this way about 470 cc. of the 784 cc. injected was recovered in the next twenty-five minutes. The flow of the fluid toward the end was faster during expiration than at other times. The dorsal roots of the spinal nerves were then exposed from the second thoracic to the second lumbar segment and divided. The animal was then turned on its back, the apparatus readjusted and the introduction of fluid into the stomach begun.

1.55 p.m. 100 cc. fluid injected. The respiration was now almost wholly diaphragmatic, the ribs moving only a little.
1.57 p.m. 100 cc. fluid injected.
1.58 p.m. 100 cc. fluid injected.
1.59 p.m. 100 cc. fluid injected.
2.01 p.m. 100 cc. fluid injected.
2.04 p.m. 78 cc. fluid injected. The pressure at which no more fluid entered the stomach was 42 cm. The respiration changed rather suddenly and became predominantly costal, the movements of the diaphragm decreasing greatly. The lever attached to the rectus abdominis now rose. See figure 2.
2.06 p.m. 50 cc. fluid injected. Pressure 61 cm.
2.07 p.m. 30 cc. fluid injected. Pressure 72 cm.
2.09 p.m. 17 cc. fluid injected. Pressure 80 cm.

Fluid allowed to escape from stomach by mouth. 345 cc. of the 675 cc. escaped.

At autopsy the stomach and intestines were found to contain saline, the intestines being very full.

To show that the lengthening of the rectus abdominis with increase in the intra-abdominal pressure is dependent upon centripetal impulses over the afferent nerves from the abdominal region, we repeated the experiments after exposing the spinal cord and dividing the dorsal roots through which the afferent nerves from the abdominal muscles enter the spinal cord. These nerves, particularly those supplying the rectus abdominis, in the cat enter through the lower thoracic roots.

Fig. 1.

Fig. 2.

Fig. 1. The upper tracing is the abdominal respiration, the second from the top the thoracic respiration, and the lower tracing the rectus abdominis muscle. Two separate amounts of fluid of 100 cc. each were introduced into the stomach during the period of time indicated by the depression of the signal (lower line), with an interval of about one minute between injections. The depression in the upper tracing indicates an increase in the abdominal girth. The abrupt drops in the lower tracing indicate a lengthening of the rectus abdominis.

Fig. 2. The order of the tracings is the same as in figure 1. The dorsal roots of the spinal nerves had been divided. 100 cc. of the fluid was introduced into the stomach during the period indicated by the depression of the signal, the slower inflow resulting from the fact that the stomach wall was approaching its limit of distention. The respiration changed rather suddenly from a predominant abdominal type to a costal type, as indicated by the relative magnitudes of excursion of the writing points of the two tambours. The rise in the curve of the rectus abdominis shows that the muscle is no longer lengthening, or at least not lengthening sufficiently to keep pace with the increase in stomach contents.

We exposed the greater part of the thoracic and the upper part of the lumbar region of the spinal cord, cutting all the dorsal roots on either side. The skin was drawn together with stitches, the animal turned on its back, and all the adjustments of stethographs and writing lever made as before. When the fluid was introduced directly into the stomach, most of it could be recovered by lowering the end of the tube through which the fluid was introduced, and the same animal could be used to show the deportment of the rectus abdominis before and after division of the dorsal nerve roots. We were not so successful in recovering the fluid from the peritoneal cavity, and had to confine ourselves to one experiment on each animal when the fluid was introduced through a hypodermic needle in the abdominal wall.

There may, at first, be some slight passive changes in the length of the rectus abdominis muscle after division of the dorsal roots of the spinal nerves and there are, of course, changes in the abdominal girth following each introduction of fluid into the peritoneal cavity, but the changes in the rectus abdominis at this early period in the experiment are neither consistent nor typical and we attribute them, in part, to the passive mechanical changes in the position of the lower ribs through distension in the abdominal cavity. These passive changes constitute a source of error in both sets of experiments, but if these changes were the only agencies involved in the lengthening of the rectus abdominis, the curves taken before section of the dorsal roots of the spinal nerves should be identical with those taken after section. They are not, however, identical as will be seen on referring to figures 1 and 2. The mere fact that the curve shown in figure 2 was made when the pressure in the stomach was significantly higher—42 cm. of salt solution—than when the curve shown in figure 1 was recorded is not the reason for the difference in the curves. When the dorsal roots are intact, the rectus abdominis continues to lengthen as long as any fluid enters the stomach. When the dorsal roots are divided, the curve of the rectus abdominis is irregular, and for the greater part of the experiment, there may be no movements of the muscle lever other than those dependent upon respiration. When the intra-abdominal pressure begins to rise, the curve indicates by a rise that the muscle is no longer lengthening, or at least, not lengthening sufficiently to compensate for the rise in intra-abdominal pressure.

The pressure at which the flow of fluid into the stomach begins to slow down or stop is not significantly different after section of the dorsal roots from the corresponding pressure observed before their division.

Before dividing the dorsal roots, the flow of fluid into the stomach stopped when the pressure fell to 37 cm. of salt solution. After division of the dorsal roots, the flow of fluid into the stomach slowed down and practically ceased when the height of the column fell to 42 cm. But the maximal distention of the stomach was not reached. By increasing the pressure of injection until the height of the column of fluid reached 80 cm., 97 cc. more fluid entered the stomach. One may infer that the dorsal roots of the thoracic region of the spinal cord have little effect on the pressure at which the walls of the stomach begin to offer resistance to the further entrance of fluid. After a second withdrawal of the fluid from the stomach and division of both vagi in the neck, resistance to a third injection of fluid was first manifested when the pressure was that of a column of salt solution 60 cm. high. This difference in pressures may have been due to the undue distention of the stomach by the previous high pressure of 80 cm., and not to the vagotomy. The division of the vagi was not repeated in any other experiment.

Water was the first fluid which we used for injecting into the stomach, but diuresis was so free that it was necessary to close the urethral orifice with a clamp in order to get the desired degree of distention of the abdominal walls. We next tried a concentrated solution of sodium chloride, on the theory that smaller amounts of a fluid whose concentration of inorganic salts would be high would be separated from the blood by the kidneys, than when water was injected into the stomach. The use of the concentrated salt solution was, however, open to some objections. At autopsy, the mucosa of the stomach and intestines was much inflamed. The presence of the concentrated sodium chloride in the intestine also induced free peristalsis. The $\frac{M}{8}$ solution of sodium chloride, with closure of the anal and urethral openings was the most satisfactory procedure.

When fluid is injected directly into the peritoneal cavity, the curve of elongation of the rectus abdominis muscle is similar in all respects to that obtained when fluid is injected into the stomach, after section of the dorsal roots of the thoracic nerves as well as when these nerve roots are intact. When the dorsal roots are intact, the fluid may enter the abdominal cavity most rapidly during expiration after the cavity begins to fill up. The rectus abdominis muscle continues to lengthen slightly, the writing point of the lever never rising, until no more fluid enters at anything like normal pressure, the pressure of injection in one experiment rising until the column of fluid was 83 cm.

The limits of extensibility of the abdominal muscles, as inferred from the pressure at which no more fluid enters, are not greatly different after section of the dorsal roots. In one experiment in which the dorsal roots of the spinal nerves were intact, the resistance to the entrance of more fluid into the peritoneal cavity appeared when the height of the column of fluid fell to 54 cm. That is, the pressure of a column of salt solution more than 54 cm. high was necessary to force any more fluid into the abdomen. In another experiment, after section of the dorsal roots, the first resistance to the entrance of more fluid was encountered when the height of the column of fluid fell to 45 cm. This agrees fairly closely with the pressure—36 cm. to 42 cm.—at which resistance to the further entrance of fluid was manifested by the stomach. Neither the abdominal wall nor the stomach withstood a pressure of much more than 80 cm. of fluid without slowly yielding, 80 cm. and 83 cm. being typical measurements. We may remark in passing that it is the muscular wall of the abdomen which limits the amount of fluid which can be introduced, and not the skin. In one animal in which the needle gradually slipped from the peritoneal cavity out through the muscle wall into the subcutaneous tissue, during the process of distention of the abdominal cavity, it was found that a relatively enormous volume of salt solution could be introduced and the posterior part of the animal assumed proportions approximating those of a football. Three liters of salt solution were in this way introduced into a young cat which weighed less than 2 kgm. before the fluid was introduced (fig. 3). How much more fluid could have been introduced we do not know. At autopsy the subcutaneous tissues resembled a mass of rather thin imperfectly hardened gelatine.

A point of some interest in connection with the mechanism of regulation of the respiratory movements came out in these experiments. Whereas, normally the thorax participates actively in the respiratory movements of the cat, it was noted that costal respiration ceased after section of the dorsal roots of the thoracic nerves. This cessation was not, however, due to any permanent paralysis of the intercostal muscles or to any anatomical injury to the ventral nerve roots. As the filling of the abdominal cavity proceeded the costal respiration was gradually resumed and at the close of the experiment was the only type of respiratory movement which was present. We may mention, also, that when the dorsal roots of the cervical nerves are divided, leaving the thoracic roots intact, it is the diaphragm which ceases to contract, while costal respiration continues uninterrupted.

We do not know the central mechanism for this adjustment of the length of the abdominal muscles to the changes in intra-abdominal pressure. Evidence has been adduced (3) that some degree of reflex tonus of the skeletal muscles of the cat persists during all phases of

Fig. 3. Upper tracing thoracic respiration, middle abdominal respiration and lower, rectus abdominis. At the time of injection of fluid into the subcutaneous tissues, there is an increase in the abdominal girth, as shown by the depression in the middle tracing, and at first, a shortening of the rectus abdominis succeeded by a slight lengthening.

spinal shock, and that a part of this mechanism must, therefore, be purely spinal. But a purely spinal mechanism does not seem sufficient for all the adjustments which occur. The change from diaphragmatic to costal respiration, or the fading out of the diaphragmatic contraction and the concomitant increase in the depth of the costal respiration almost certainly involve a central mechanism as high up as the medulla oblongata. We are inclined to regard these changes in length of the rectus, and probably of the other abdominal muscles, as part of the respiratory movements, and to be under the control of the respiratory nervous mechanism.

The abdominal muscles may be regarded, in one sense, as antigravity muscles, since they sustain a fairly constant weight of viscera whenever the animal is in a normal posture for locomotion. The question arises whether their postural changes are dependent in any degree upon the cerebellum. We hope to present further data on this and other questions concerning the central mechanism.

We believe that the experiments show that the regulation of the length of the abdominal muscles corresponding to the increase in volume of the contents of the abdominal cavity is a reflex process dependent upon afferent impulses, and that it falls into line with the other known instances of postural activity of muscle and nerve. We may recall here Langley's (4) view that there is a receptive substance in muscle capable of causing a slow contraction, as well as a receptive substance for a quick contraction, and Lingle's (5) observations on the histological appearance of striated muscles. In whatever phase the excised muscle was fixed, Lingle noticed that there were some nuclei which indicated by their shape that the cells to which they belonged were partially or wholly contracted, while other nuclei in adjacent fibers might indicate an almost complete lack of such contraction. It seems probable that the abdominal muscles, which normally undergo considerable changes in length during the life of the individual, must, at all times, have some fibers in a state of contraction, while others are partly or wholly relaxed. Variation in the number of the contracted fibers might be expected to correspond to the degree of passive extension of the muscles.

BIBLIOGRAPHY

(1) SHERRINGTON: Brain, 1915, xxxviii, 191–233.
(2) STEWART: Manual of Physiology, 6th Ed., New York, 1910, 289.
(3) PIKE: Quart. Journ. Exper. Physiol., 1912, vii, 1–29.
(4) LANGLEY: Journ. Physiol., 1907–1908, xxxvi, 348.
(5) LINGLE: This Journal, 1910, xxvi, 361.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XL. The Ammonia of the Gastric Juice

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A review of the literature on the chemistry of the gastric juice shows that the findings as to the amount of ammonia and the views as to the origin and significance of this ammonia vary greatly. This discrepancy in findings is no doubt due partly to the difference in character of material analyzed and partly to the different methods of analysis used. The importance of the analytical method which is used is clearly shown by Folin (9) in his work on the ammonia content of the portal blood. A brief summary of some of the work on gastric juice ammonia follows:

OBSERVER	DATE	METHOD OF ANALYSIS	MATERIAL USED	RESULTS
Bidder and Schmitt (2)	1852		Dog with gastric fistula	Trace of ammonia found
Bidder and Schmitt (2)	1852		Man	No trace found
Leo (22)	1892	Schlösing	Man; Ewald meal	Trace of NH_3 in health. More in uremic cases
Rosenheim (30)	1892	Schlösing	Man	Trace of NH_3 as NH_4Cl. Comes from gastric secretion or from decomposition of albumen.
Martius and Lüttecke (24)	1892		Man	NH_3 found only as NH_4Cl in cases of uremia.
Ewald (7)	1892		Man	NH_3 found in 50 per cent of cases examined

OBSERVER	DATE	METHOD OF ANALYSIS	MATERIAL USED	RESULTS
Strauss (36)	1893	Strauss	Man; gastric fistula and gastric disorders	NH_3 in 80 per cent of cases examined
Sticker (35)	1896	Schlösing and Nessler	Man; test meal	NH_3 in gastric juice comes from saliva or from decomposed albumen in stomach disorders
Salaskin (31)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Nencki-Pavlov-Zaleski (26)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Horodynski-Salaskin-Zaleski (17)	1898	Nencki-Zaleski	Dog; stomach contents	High NH_3 content
Reissner (27)	1903		Dog with Pavlov pouch	NH_3 when found comes from saliva or from decomposed albumen
Sommerfeld (34)	1905		Man with gastric fistula	No trace of NH_3
Rosemann (29)	1907	Schlösing	Dog; esophageal fistula	NH_3 present comes from secretions of stomach
Zunz (40)	1910		Man after test meals	NH_3 present; more abundant in malignancy.
Wolf and Junghaus (39)	1911		Ewald meal	Soluble protein found increased in cases of malignancy
Carlson (3)	1915	Folin-Nessler	Dog; Pavlov pouch	NH_3 in all cases
Carlson (3)	1915	Folin-Nessler	Man: gastric fistula	NH_3 in all cases
Carlson (3)	1915	Folin-Nessler	Man; Rehfus tube	NH_3 in all cases

In an effort to clear up some of the points of difference brought out by this comparison of data and to determine more accurately the factors which influence the amount of gastric juice ammonia, this experimental work was carried out, at the suggestion of Dr. Carlson, on normal and pathological men and dogs. The work is an attempt to throw further light on the following questions:

1. Is ammonia normally excreted from the blood by the gastric mucosa?
2. Is it produced in part by deaminization of amino-acids in the gastric mucosa?
3. Is it a necessary by-product of the normal secretion processes of the gastric mucosa?
4. Is it due to the action of enzymes or the HCl on the gastric juice proteins or on the mucous surface?
5. Is it due to action of bacteria in the stomach?
6. What is its pathological significance?

The ammonia in the pure gastric juice of normal dogs

DOG NO.	MATERIAL	NUMBER OF OBSERVATIONS	NH_3 IN MGM. PER 100 CC. GASTRIC JUICE			AVERAGE ACIDITY	
			Maximum	Minimum	Average	Free	Total
1	1 hour before	13	5.0	0.5	1.7	0.000	0.0093
	1 hour after	14	8.0	0.5	2.1	0.0996	0.1708
	Second hour after	6	6.0	0.5	2.6	0.2598	0.2910
2	1 hour before	6	8.0	2.0	5.0	0.0045	0.0205
	1 hour after	7	5.0	3.0	3.7	0.1029	0.1466
	Second hour after	4	6.0	2.5	4.0	0.2881	0.2903
3	1 hour before	3	5.0	3.5	4.1	0.1029	0.1993
	1 hour after	3	4.0	2.5	3.3	0.2685	0.3054
	Second hour after	2	2.5	2.5	2.5	0.2679	0.3965
4	1 hour after	3	3.5	1.5	2.8	0.1845	0.2393
5	1 hour after	1	2.5	2.5	2.5	0.0729	0.1641
6	1 hour before	2	6.0	2.0	4.0		
7	†1 hour after injection	2	2.5	2.5	2.5	0.2361	0.3085
8	1 hour before	2	2.0	1.5	1.75	0.1995	0.2989
	1 hour after	2	3.0	2.0	2.5	0.2644	0.3209
	2 hours after	2	4.0	2.5	3.25	0.2829	0.3554
	*1 hour before	3	2.0	1.5	1.8	0.000	0.0392
	1 hour after	3	3.5	0.5	2.8	0.1185	0.1905
	2 hours after	3	6.0	2.0	4.0	0.3664	0.4119
9	1 hour before	4	6.0	2.0	4.4	0.1130	0.1840
	1 hour after	4	5.0	3.0	3.8	0.1258	0.1813
	2 hours after	4	6.0	2.5	4.2	0.3746	0.4247
	*1 hour before	4	6.0	2.0	4.5	0.000	0.0547
	1 hour after	4	12.0	4.0	8.7	0.2280	0.2809
	2 hours after	4	12.0	8.0	10.0	0.4220	0.4730

* 5 gm. NH_4Cl were added to the regular diet.
† Gastrin injected.

THE AMMONIA OF THE GASTRIC JUICE OF DOGS

The gastric juice used in these determinations was collected from the accessory stomach pouch prepared according to the method of Pavlov. The collections were made in the morning for an hour period before feeding and for hour periods after feeding, the character and amount of food being a fixed constant during the entire observation period. The method of collecting the juice was the same as that used by Hardt (15) in his work on experimental ulcers in dogs. The ammonia was separated from the fresh juice by the Folin (8) aeration method and the amount determined by the Nessler colorimetric method as modified by Gulick (14). The results on normal dogs are summarized in table 1.

This table shows that the ammonia content of the pure gastric juice of normal dogs varies greatly in different dogs and in the same dog at different times. These findings are in accord with those recently reported by Carlson (3). In dogs that were fed a protein diet there was no uniform increase in ammonia content during the digestion period even in secretions collected from four to seven hours after feeding. When small amounts (5 gram) of NH_4Cl were added to the protein diet there was an increase in the ammonia content in most cases. This increase was usually most marked during the second and third hour after feeding.

THE AMMONIA IN THE GASTRIC JUICE OF DOGS WITH VARIOUS PATHOLOGICAL CONDITIONS

TABLE 2

The ammonia in the gastric juice of pathological dogs

DOG NO.	MATERIAL	NO. OF OBSERVATIONS	NH_3 IN MGM. PER 100 CC.			ACIDITY		
			Maximum	Minimum	Average	Free	Total	
10	Hunger juice	2	4.0	0.5	2.2	0.2630	0.3336	Distemper (sniffles)
11	Hunger juice	4	0.5	0.2	0.3	0.3190	0.3992	Pancreatectomy
12	Hunger juice	1	16.0	16.0	16.0			Pancreatectomy plus acute gastritis
13	Hunger juice	4	3.0	1.5	2.1	0.1595	0.2325	Splanchnics cut
14	Hunger juice	5	48.0	3.0	27.0	0.2771	0.4048	Ulcer in pouch
15	Hunger juice	7	32.0	12.0	20.0	0.1230	0.2278	Ulcer in pouch
	Second hour	9	36.0	12.0	19.0	0.2535	0.3319	
	Third hour	3	40.0	18.0	26.0	0.3326	0.4310	

At autopsy Dog 12 showed a very hyperemic gastric mucosa but no definite ulcer. Dog 14 was used by Mr. L. L. Hardt (15) in work on experimental gastric ulcer. On the first day of observation the total acidity was 0.3144 per cent and the NH_3 content 3 mgm. per 100 cc. of juice. Three days later the dog showed signs of distemper and refused to eat. The acidity at that time was 0.4174 per cent and the NH_3 content 24 mgm. Juice collected one hour before death showed an acidity of 0.3921 per cent and an NH_3 content of 48 mgm. At autopsy a definite ulcer was found in the Pavlov pouch.

Dog 13 also was used by Mr. L. L. Hardt in his gastric ulcer work. The first determinations thirty-two days before death showed a total acidity of 0.3644 per cent and an NH_3 content of 12 mgm. per 100 cc. of juice. Several days before being killed this dog refused to eat and from that time on there was a progressively increasing NH_3 content while the acidity remained at the same level. The day of death the total acidity was 0.4310 per cent and the NH_3 content was 40 mgm. At autopsy the Pavlov pouch showed a distinct almost healed ulcer but no other lesions could be found.

THE AMMONIA IN THE GASTRIC JUICE OF NORMAL MEN

The gastric juice used in these determinations was collected, except from Mr. V., the gastric fistula case, by means of the Rehfus (28) stomach tube. Three collections were usually made: first, the empty stomach; second, one-half hour of continuous secretion; third, one hour after taking an Ewald meal consisting of two pieces of dry toast and 200 cc. of distilled water. The determinations were made on samples from these collections by the method described above, except that in the Ewald meal collection a filtration was necessary before aeration could be satisfactorily accomplished. The findings for normal gastric juice are given in table 3.

All of these men were to the best of their knowledge in perfect health and all except V. and C. used the Rehfus tube on themselves almost daily in another line of experiments. A comparison of these results with the results found in normal dogs shows similar variations, but the findings are somewhat higher in man. In each case the Ewald meal juice shows the highest acidity and the lowest ammonia content.

TABLE 3

The ammonia in the gastric juice of normal men

INDIVIDUAL	MATERIAL	NO. OF OBS.	FREE ACIDITY	TOTAL ACIDITY	NH_3
			per cent	*per cent*	
Mr. V.	Hunger juice	3	0.1643	0.2285	0.5
	Continuous juice	4	0.2914	0.3826	3.5
	Appetite juice	4	0.3642	0.4106	2.5
Mr. D.	Hunger juice	5	0.0228	0.0729	12.0
Mr. H.	Hunger juice	6	0.1897	0.2380	9.0
	Continuous	6	0.1979	0.2371	9.0
	Ewald	6	0.2152	0.2544	4.0
Mr. M.	Ewald	3	0.1826	0.2006	3.0
Dr. E.	Ewald	3	0.0738	0.1322	0.5
Mr. L. D.	Ewald	2			3.0
Mr. L. H.	Hunger juice	2	0.0784	0.1240	3.0
	Ewald	2	0.2169	0.2716	1.5
Dr. C.	Hunger juice	3	0.0273	0.0547	16.0
	Continuous	4	0.0000	0.0474	14.0
	Ewald	3	0.0064	0.0711	12.0

THE AMMONIA IN THE GASTRIC JUICE OF MAN IN GASTRO-INTESTINAL DISEASES

The gastric juice employed in this work was collected with the ordinary stomach tube from individuals with gastric disturbances. The collections were made in the morning: first, aspiration of the empty stomach; second, aspiration one hour after taking an Ewald test meal consisting of two pieces of bread and 200 cc. of water. The ammonia in these samples was determined by the method described above. Observations were made in some of the individuals at the time of entrance into the hospital and in others after they had been on the ulcer treatment as outlined by Sippy (33) for a short time. Table 4 shows the average result of the findings with the probable or definite diagnosis in each individual case.

In all cases except one (22) the acidity of the Ewald meal juice exceeded the acidity of the plain aspiration juice but the ammonia content had no constant relation to the acidity and was not altered by a long stay in the hospital under treatment. All patients who had a relatively high NH_3 content for a period of several days were found to have either an ulcer or a carcinoma of the stomach.

TABLE 4

The ammonia in gastric juice of man in gastro-intestinal disorders

HISTORY	NO. OF OBSERVATIONS	MATERIAL	ACIDITY		NH_3
			Free	Total	
1. Mr. K. Age 26. Entered on April 29.	7	Aspiration	0.0552	0.1221	1.1
Observed from May 9–26. Diagnosis, peptic ulcer	4	Ewald	0.1744	0.2655	1.1
2. Mr. L. Age 33. Entered April 12.	3	Aspiration	0.0350	0.0847	1.0
Observed from May 9–13. Diagnosis, peptic ulcer	2	Ewald	0.1906	0.2703	1.2
3. Dr. B. Age 25. Entered April 20.	2	Aspiration	0.0301	0.0637	1.2
Observed from May 9–11. Diagnosis, peptic ulcer, tooth abscess	1	Ewald	0.1960	0.2507	1.5
4. Mr. N. Age 31. Entered March 30.	3	Aspiration	0.0926	0.1361	2.2
Observed from May 9–26. Diagnosis, peptic ulcer, alveolar abscess. Roentgen diagnosis, duodenal ulcer	3	Ewald	0.1966	0.2766	2.5
5. Mr. L. Age 53. Entered April 14.	6	Aspiration	0.1319	0.2227	0.5
Observed from May 17–29. Diagnosis, peptic ulcer	4	Ewald	0.2079	0.2779	0.5
6. Mr. A. Age 38. Entered May 10.	7	Aspiration	0.0636	0.1798	1.7
Observed from May 13–31. Diagnosis, peptic ulcer	6	Ewald	0.1527	0.2267	0.7
7. Miss B. Age 21. Entered May 7.	1	Aspiration	0.0136	0.0637	0.5
Observed from May 29–June 4. Diagnosis, peptic ulcer	3	Ewald	0.1857	0.2534	1.7
8. Miss G. Age 20. Entered May 23.	2	Aspiration	0.0781	0.1209	0.5
Observed from June 2–4. Diagnosis, peptic ulcer	1	Ewald	0.1230	0.2506	0.5
9. Mr. N. Age 47. Entered June 1.	4	Aspiration	0.1558	0.1955	0.6
Observed June 7–9. Diagnosis, peptic ulcer	1	Ewald	0.1851	0.2598	1.0
10. Mr. A. Age 31. Entered June 1. Observed June 7. Diagnosis, peptic ulcer	2	Aspiration	0.1882	0.2585	1.5
11. Mr. C. Age 34. Entered May 13. Observed June 8. Diagnosis, peptic and duodenal ulcers	1	Aspiration	0.0683	0.1321	0.2

TABLE 4—Continued

HISTORY	NO. OF OBSERVATIONS	MATERIAL	ACIDITY		NH_3
			Free	Total	
12. Mr. K. Age 23. Entered May 27. Observed June 7. Diagnosis, peptic ulcer	1	Aspiration	0.1532	0.2416	4.0
13. Mr. R. Age 46. Entered May 24. Observed May 26. Diagnosis, peptic ulcer	1	Aspiration	0.0820	0.1321	6.0
14. Mr. S. Age 42. Entered May 13. Observed May 26–30. Diagnosis, peptic ulcer with obstruction. Gastroenterostomy, ulcer found	2	Aspiration	0.00	0.1180	16.0
15. Mr. G. Age 27. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1750	0.2744	12.0
16. Mr. C. Age 32. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1641	0.2452	1.0
17. Mr. H. Age 35. Entered June 15. Observed June 16. Diagnosis, peptic ulcer	1	Ewald	0.1732	0.2981	2.0
18. Mr. M. Age 21. Entered June 16. Observed June 17. Diagnosis, peptic ulcer	1	Ewald	0.0985	0.2353	3.0
19. Mr. P. Age 46. Entered June 15. Observed June 17. Diagnosis, tabes	1	Ewald	0.1167	0.3054	12.0
20. Mr. E. Age 20. Entered April 8. Observed May 17. Diagnosis, tuberculosis of bronchial glands	1	Ewald	0.2170	0.2735	1.5
21. Mr. F. Age 66. Entered June 7. Observed June 8. Diagnosis, carcinoma of stomach	1	Ewald	0.00	0.0695	1.0
22. Mrs. B. Age 42. Entered April 28. Observed May 9–20. Diagnosis, carcinoma of stomach, confirmed by operation	4 4	Aspiration Ewald	0.0408 0.0121	0.0788 0.0321	4.9 4.6
23. Mrs. M. Age 59. Entered. Observed July 7. Diagnosis, carcinoma of stomach	4	Aspiration	0.00	0.0604	13.0

THE INFLUENCE OF VARIATIONS IN THE DIET ON THE AMMONIA OF GASTRIC JUICE

In order to determine some of the factors that influence the NH_3 content of the gastric juice a series of experiments was conducted on one of the normal individuals (Mr. H.), who showed a constant high NH_3 content. During these experiments the gastric juice was collected twice each morning before breakfast. The stomach was first emptied with the Rehfus tube, then washed thoroughly with distilled water and emptied again. A second collection was made after one-half an hour of continuous secretion. The free and total acidity and the NH_3 content of both collections were then determined. In order to determine the relation of the NH_3 content of the gastric juice to the NH_3 content of the urine, determinations of the NH_3 content and of the total N of the urine were made on the total urine for each day. The NH_3 of the urine was determined by the method described above and the total N was determined by the Kjeldahl-digestion-distillation-titration method.

In the first experiment the diet was so arranged that there was a period of high protein and a period of low protein ingestion. In the second experiment the diet was kept uniform but during certain periods an excess of base- or of acid-forming radicals was added in water solutions. The base-forming radicals were increased by using the treatment for gastric ulcer as outlined by Sippy (33). The acid-forming radical was increased by adding phosphoric acid, which is a normal constituent of the protein molecule. The complete results of the two experiments are recorded in tables 5 and 6.

The low protein diet consisted of potatoes, rice, fruit, cream and a small amount of bread. There were no noticeable symptoms during the period of ingestion of this diet.

The high protein diet consisted almost wholly of meats—fish, steak, liver, sweetbreads, etc. with a small allowance of bread. No fats were ingested. The symptoms during this period were very noticeable; first day, nothing noticeable; second day, easily fatigued on exertion; third day, easily fatigued on slight exertion, joints and muscles aching, could work only a short time without rest. On this day the urine contained many uric acid crystals; fourth day, symptoms of third day more pronounced. Very sleepy and depressed. Urine contained a heavy precipitate of uric acid.

These symptoms cleared up quickly when the mixed diet was resumed. The absolute increase or decrease in the urinary NH_3 follow-

TABLE 5

The influence of variation in protein content of diet on the ammonia of the gastric juice

DATE	GASTRIC JUICE			URINE			
	Acidity		NH_3	Volume	Total N	Total NH_3	NH_3
	Free	Total					
			Ordinary diet				
						mgm.	*per cent*
July 10.....	0.0638	0.1094	16	800	4.1664	320.0	7.69
July 11.....	0.0665	0.1020	16	650	3.735	292.5	7.83
			Low protein diet				
July 12.....	0.1066	0.1713	8	680	3.843	258.4	6.71
July 13.....	0.1148	0.1494	7	610	4.833	129.6	2.66
July 14.....	0.1531	0.2078	5	1620	5.493	162.0	3.71
July 15.....	0.2598	0.2871	3.5	760	6.719	243.0	3.62
			Ordinary diet				
July 16.....	0.0337	0.0820	12	960	10.666	384	3.60
July 17.....	0.1395	0.2033	12	680	9.203	380.8	4.13
July 18.....	0.1021	0.1349	14	895	11.335	319.2	2.81
July 19.....	0.0729	0.1258	16	1080	11.83	361.8	3.06
July 22.....	0.1303	0.1740	10	990	9.41	356.4	3.78
			High protein diet				
July 23.....	0.1167	0.1659	10	1155	19.36	369.6	1.905
July 24.....	0.1395	0.1851	29	1420	24.54	426.0	1.734
July 25.....	0.1003	0.1413	24	1530	27.86	550.8	1.977
July 26.....	0.1321	0.1658	26	1510	24.04	815.0	3.388
			Ordinary diet				
July 27.....	0.0656	0.1002	19	1110	14.02	799.2	5.970
July 28.....	0.0747	0.1148	12	1500	9.91	600.0	6.053
July 29.....	0.0638	0.1003	10	1365	9.86	435.0	4.411

ing increase or decrease in the protein content of the diet is more marked than is usually observed. We are unable to give a satisfactory explanation for this variation.

TABLE 6

The influence of variations in acid and basic-radical content of diet on the ammonia of gastric juice

DIET	DATE	GASTRIC JUICE			URINE			
		Acidity		NH_3	Volume	Total N	Total NH_3	NH_3
		Free	Total					
								per cent
Ordinary diet.....	August 15	0.0610	0.1003	16	800	9.576	394.32	4.11
	August 16	0.0182	0.0547	14	1000	11.599	448.8	3.87
	August 17	0.0783	0.1211	10	1215	9.460	427.6	4.52
Ordinary diet plus alkali. Per day: 50 gr. calcined Mg.; 150–300 gr. $NaHCO_3$; 75 gr. bismuth subcarbonate; Bi_2O_3-CO_2H_2O.....	August 18	0.1368	0.1687	10	1570	8.715	415.6	4.76
	August 19	0.1185	0.1659	10	1715	9.24	429.0	4.64
	August 20	0.1832	0.2260	9	1705	8.21	351.0	4.27
	August 21	0.0939	0.1349	10	1470	8.448	237.0	2.80
	August 22	0.1686	0.1956	12	2035	8.632	105 4	1.22
	August 23	0.0519	0.0820	14	1365	8.738	40.2	0.46
Ordinary diet.....	August 24	0.0747	0.1048	12	1220	8.569	106.75	1.24
	August 25	0.0620	0.0866	12	1260	10.900	146.28	1.34
	August 26	0.1777	0.2296	10	1530	15.492	432.32	2.98
	August 27	0.0547	0.0866	12	2135	10.259	339.7	3.31
	August 28	0.0865	0.1138	12	1290	9.476	360.0	3.97
	August 29	0.0912	0.1185	12	1050	10.574	285.6	2.70
	August 30	0.1003	0.1413	13	1580	11.336	364.2	3.21
	August 31	0.2006	0.2325	12	1280	9.873	375.6	3.79
Ordinary diet plus acid. Per day: 15 to 20 cc. of a 4 per cent syrup of phosphoric acid.....	September 1	0.1230	0.1458	9	1315	9.336	333.6	3.48
	September 2	0.1021	0.1322	10	1635	8.402	310.8	3.69
	September 3	0.1641	0.1987	11	1070	7.179	414.3	5.77
Ordinary diet.....	September 4	0.0838	0.1157	12	1295	9.590	368.3	3.83

During the period of ingestion of a large excess of alkali radicals there were no noticeable symptoms but the total acidity of the urine was greatly diminished. During the period of ingestion of excess of

acid radicals there was a feeling of discomfort for two hours after taking each dose. No other symptoms were noted. On the last day of this acid-radical ingestion the total acidity of the urine was markedly increased.

In all of the work on normal or pathological man the juice collected frequently contained varying amounts of bile but the NH_3 content of these samples did not seem to be influenced.

THE AMMONIA CONTENT OF THE GASTRIC MUCOSA

Salaskin (31), Horodynski, Salaskin and Zaleski (17) by using the Nencki-Zaleski method of NH_3 determination found a high percentage of NH_3 in the contents and mucosa of the stomach and intestines of animals. As the presence of this relatively high percentage of NH_3

TABLE 7.

NH_3 content of gastric and intestinal mucosa in dogs. Per 100 grams fresh mucosa

DOG	I	II	III	IV	V	AVERAGE	AMOUNT FOUND BY ZALESKI-NENCKI AND PAVLOV
Hours without food	2	15	18	19	45		
NH_3 mgm. in 100 grams cardiac mucosa	0.60	1.41	0.50	0.46	0.22	0.64	
NH_3 mgm. in 100 grams pyloric mucosa	1.78	2.65	1.67	0.62	1.04	1.55	37.1–52.8
NH_3 mgm. in 100 grams fundus mucosa	1.2	4.63	5.25	1.40	5.22	3.54	
NH_3 mgm. in 100 grams duodenal mucosa	1.1			0.94	0.90	0.97	23.0–41.7

Nencki, Pavlov and Zaleski made their determinations on the mucosa from animals that had been fed two to nine hours before being killed. Horodynski, Salaskin and Zaleski obtained approximately the same results.

in both the contents and mucosa of these organs suggested a connection with their secretory processes some NH_3 determinations were made by the newer methods on these tissues in dogs. Four dogs that had been fasted from fifteen to forty-five hours, and one that had been fed only two hours previously, were killed by bleeding. The stomach and duodenum were immediately removed, opened and washed thoroughly with distilled water. Eight to ten grams of the mucosa was stripped from the cardiac, fundic and pyloric regions of the stomach and from the duodenum, and these portions of mucosa were pulverized in a mortar with purified sea sand. These pulverized masses were extracted several times with warm distilled water which was slightly

acidulated with acetic acid, the washings for each portion combined, boiled for five to ten minutes, and filtered. The acid filtrate was evaporated on the steam bath to about 15–20 cc. and the NH_3 content determined by the methods described above.

DISCUSSION

The data presented above shows that ammonia is a constant constituent of the gastric juice of man and dog and that the amount found varies between rather wide limits, especially in man. Many investigators, Sticker (35), Sommerfeld (34), Reissner (27) and others, have contended that this ammonia has its origin in the saliva and Boldyreff (1) has shown that the duodenal content is often regurgitated into the stomach. Strauss (36), and more recently Carlson (3) eliminated one or both of these possible sources of contamination by using the juice collected from men with a gastric fistula or from dogs with a Pavlov stomach pouch. Their work showed that ammonia was constantly present in pure gastric juice. Rosenheim (31) concluded that the ammonia came from the secretions of the stomach itself as it was present in approximately the same amount regardless of the time of test or state of digestion. Rosemann (29) thought that the ammonia must be a product of cell activity as it was in higher concentration in the gastric juice than in the blood. Zunz (40) found by analyzing stomach contents and test meals that there was no relation between the amount of ammonia and the state of digestion or the concentration of the hydrochloric acid. The present work is in accord with and corroborates these findings of Strauss, Carlson, Rosenheim and Zunz and shows further, that the ammonia content of the gastric juice of man may be increased or diminished by varying the protein content of the diet. During this variation in diet there is a corresponding variation in urinary ammonia. Loeb (23), Schittenhelm (32), Grammeltoft (13) and others, have shown that urinary ammonia decreases in amount during the height of gastric secretion. Although these facts seem to point to a possible relation between the occurrence of ammonia in the urine and in the gastric juice, the controlling mechanisms are influenced differently by at least one factor, i.e., the ingestion of an excess of acid- or base-forming radicals. In this case the ingestion of an excess of base-forming radicals causes a marked decrease in urinary ammonia and the ingestion of an excess of acid-forming radicals has the opposite effect, but neither of these has any

effect on the amount of gastric juice ammonia. It was also noted that in man during a period of fasting lasting from twenty-four to forty hours the ammonia content of the gastric juice decreased progressively, but that during the height of absorption of a mixed or protein diet in dogs there was no corresponding increase except in some cases where NH_4Cl was added to the diet. However, this last observation is not altogether trustwortny as Underhill and Goldschmidt (37) have found that "the ingestion of ammonium chloride may be regarded as a distinct detriment to nutritional rhythm" and they believe that ammonium chloride acts as a stimulant to protein katabolism.

During recent years much work has been done on the fluids originating in the gastro-intestinal tract and some of these observations are of especial interest in the present work. It has been shown by different workers that the mucosa of the alimentary tract may act as an excretory organ for such substances as Fe, Ca, PO_4, Hg, various pigments, mono-saccharides, etc.[1] Voit (38) and Hermann (16) were among the first to show the importance of the nitrogen content of the fluids collected from the alimentary tract. Corlette (5) concluded that the excretion of nitrogen from the bowel wall did not vary according to general metabolism in the same way as occurred in the kidneys. This observation is in accord with our results. Mosenthal (25) while studying the secretion of a Thiry fistula of the lower ileum in dogs estimated that the total nitrogen secreted by the small intestine varied between 225 to 607 per cent of that found in the feces, the greater part of this apparently being reabsorbed. Of this nitrogen, about 1.5 to 3.25 per cent was in the form of ammonia. He states further that the intestine has at least three functions, namely; excretory, digestive including absorption, and a third, that of forming a link in the "circulation" of various products, such as nitrogen or the bile pigments. We made some ammonia determinations on juice collected from a Thiry fistula of different parts of the small intestine of dogs and found that the ammonia ranged between 1.5 to 12 mgm. per 100 cc. of juice.

Another point of interest in this connection is the observation made by Cohnheim (4) that the intestinal mucosa of fishes has the power to deaminize proteins. However, he was unable to extend this observation to mammals although Jacobi (18) and Lang (21) had shown that tissue extracts of the intestine were able to deaminize amino-acids.

[1] The occurrence of ulcers in the duodenum following extensive superficial burns and in the colon in the late stages of uremia gives further evidence of this excretion.

In Folin's (12) original theory of protein metabolism the assumption is made that the intestinal mucosa has the power of deaminization. Abderhalden favors the view that the protein molecule is broken down completely in the intestinal mucosa. Later, Folin (9) concluded that the intestinal mucosa had no special deaminizing power for he was able to show that the ammonia content of the portal blood was directly dependent on the putrefactive processes in the large intestine. Folin (10) also showed definitely that there is an absorption of protein digestion products by the stomach mucosa. It should be noted that the workers on the mechanism of intestinal absorption and metabolism have taken into account only those products found in the efferent blood or lymph vessels and have neglected altogether those products which pass back into the lumen of the intestine.

There are certain factors which speak against considering the gastric juice ammonia as due to either a normal secretory or excretory process. Although ammonia is constantly found in all samples of normal gastric juice, it is evident that the mechanism governing the production of this constituent is not the same as that governing the acid or pepsin production, for the concentration of the ammonia has no relation to the concentration of the acid or pepsin, and there seems to be no relation between the ammonia and the amount of mucus. The similar variations in concentration of urinary and gastric juice ammonia which may be brought by varying the N-content of the diet seem to point to an excretory process, but the mechanisms controlling these processes are different, for the urinary ammonia may be made to vary markedly by other means without affecting the concentration of the gastric juice ammonia.

Zunz (40), working on stomach contents and test meals, showed that in some pathological cases the ammonia nitrogen was appreciably increased but he was unable to account for this increase. A few years before Emerson (6) demonstrated an enzyme in malignant growths which was capable of splitting protein beyond the albumose stage. Jacque and Woodyatt (19) also showed that carcinomatous stomach contents had, in 88 per cent of cases examined, a peptolytic power two to ten times the maximum seen in benign conditions and suggest that a part of this is due to bacteria, as the degree of proteolysis was lowered somewhat by filtration through a Berkefeld filter. Folin, as was noted above, showed a definite relation between ammonia content of the portal blood and bacterial decomposition in the large intestines. Recently, Burgett has isolated a fairly constant flora from the pure

gastric juice of Mr. V., the gastric fistula case. Kendall and Farmer (20) have reported that many bacteria have a deaminizing power which is exercised to a marked degree if carbohydrate is lacking or small in amount.

It seems then that enzymatic or bacterial action on the stomach mucosa or on the gastric juice may be responsible for some of the ammonia found in the gastric juice in some cases, but the constancy of the ammonia content of the fresh juice of normal man and dog speaks against this as the sole or principal origin.

The diagnostic significance of a high content of ammonia in the gastric juice in cases of ulcer or gastric cancer is made questionable by the finding of an equally high content in the juice of some apparently normal individuals.

It was observed that some of these cases (hospital patients and sick dogs) with high ammonia values showed a marked cachexia. Observations are being made to determine whether any relation exists between cachexia, acidosis and the ammonia content of the gastric juice. Folin's (11) new method of NH_3-determinations by direct Nesslerization is being used in this work.

CONCLUSIONS

1. Ammonia is normally found in small amounts in the gastric juice of man and dog.

2. The amount of ammonia in the pure gastric juice of man varies markedly in different individuals. It is increased by high protein diet, and by the addition of ammonium salts to the food.

3. The fundic mucosa contains the most ammonia, the pyloric mucosa is next in order, while mucosa of the cardiac end of the stomach contains the least ammonia. The duodenal mucosa contains less ammonia than the fundic mucosa.

4. In certain pathological conditions (especially gastric ulcer and cancer) the amount of ammonia in the gastric juice is definitely increased, but this increase may not be greater than that found persistently in certain normal individuals.

5. Our results indicate that the origin of the gastric juice ammonia is a complex one. Some of the ammonia is evidently excreted from the blood, and this factor depends on the concentration of the ammonia in the blood. Part of the ammonia represents deaminization processes in the gastric mucosa, and these in turn depend on the quan-

tity of protein in the food. The possible rôle of the gastric flora has not been eliminated.

NOTE.—I wish to thank Drs. Carlson and Koch for their many helpful suggestions, also members of the university laboratories and of the different hospital staffs for their coöperation in obtaining samples of gastric juice.

BIBLIOGRAPHY

(1) BOLDYREFF: Quart. Journ. Exper. Phys., 1914, viii, 1.
(2) BIDDER UND SCHMITT: Verdauungssäfte u. d. Stoffwechsel, 1852.
(3) CARLSON: This Journal, 1915, xxxviii, 248.
(4) COHNHEIM: Zeitschr. f. physiol. Chem., 1909, lix, 239; 1909, lxi, 189; 1912, lxxvi, 293.
(5) CORLETTE: Journ. Physiol., 1899, xxv, 344.
(6) EMERSON: Deutsch. Arch. f. klin. Med., 1902, lxxii, 415.
(7) EWALD: Zeitschr. f. klin. Med., 1892, xx, 534.
(8) FOLIN: Journ. Biol. Chem., 1912, xi, 523.
(9) FOLIN: Journ. Biol. Chem., 1912, xi, 161.
(10) FOLIN: Journ. Biol. Chem., 1912, xii, 259.
(11) FOLIN: Journ. Biol. Chem., 1916, xxvi, 497.
(12) FOLIN: This Journal, 1905, xiii, 117.
(13) GAMMELTOFT: Zeitschr. f. physiol. Chem., 1911, lxxv, 57.
(14) GULICK: Journ. Biol. Chem., 1914, xviii, 541.
(15) HARDT: This Journal, 1916, xl, 314.
(16) HERMANN: Arch. f. d. gesammt. Physiol., 1890, xlvi, 93.
(17) HORODYNSKI, SALASKIN UND ZALESKI: Zeitschr. f. physiol. Chem., 1902, xxxv, 246.
(18) JACOBI: Zeitschr. f. physiol. Chem., 1900, xxx, 149.
(19) JACQUE AND WOODYATT: Arch. Int. Med., 1912, x, 560.
(20) KENDALL AND FARMER: Journ. Biol. Chem., 1912, xiii; 1913, xv, 277.
(21) LANG: Beitrag. z. Chem. Phys. u. Physiol., 1904, v, 321.
(22) LEO: Deutsch. med. Wochenschr., 1891, xvii, 1145.
(23) LOEB: Zeitschr. f. klin. Med., 1905, lvi, 100.
(24) MARTIUS UND LÜTTCKE: Die Magensaure d. Menschens, 1892.
(25) MOSENTHAL: Journ. Exper. Med., 1911, xiii, 319.
(26) NENCKI, PAVLOV UND ZALESKI: Arch. f. exper. Path. u. Pharm., 1898, xxxvii, 26.
(27) REISSNER: Zeitschr. f. klin. Med., 1903, xlviii, 101.
(28) REHFUS: Amer. Journ. Med. Sci., 1914, cxlvii, 848.
(29) ROSEMANN: Arch. f. d. gesammt. Physiol., 1907, cxviii, 467.
(30) ROSENHEIM: Centralbl. f. klin. Med., 1892, xiii, 817.
(31) SALASKIN: Zeitschr. f. physiol. chem., 1898, xxv, 449.
(32) SCHITTENHELM: Deutsch. Arch. f. klin. Med., 1903, lxxvii, 517.
(33) SIPPY: Journ. Amer. Med. Assoc., 1915, lxiv, 1625.
(34) SOMMERFELD: Arch. f. (Anat. u.) Physiol. Suppl., 1905, 455.
(35) STICKER: Münch. med. Wochenschr., 1896, xliii, 1041.

(36) Strauss: Berl. klin. Wochenschr., 1893, xxx, 398.
(37) Underhill and Goldschmitt: Journ. Biol. Chem., 1913, xv, 341.
(38) Voit: Zeitschr. f. Biol.,.1892, xxix, 325.
(39) Wolf und Junghaus: Berl. klin. Wochenschr., 1911, xxii, 978.
(40) Zunz: Intern. Beitr. z. Pathol. u. Therap. d. Ernahrungsstörungen, 1910, ii, 372.

VI. DIFFERENCES IN LATENT PERIOD AND FORM OF THE CONTRACTION CURVE IN MUSCLE STRIPS FROM DIFFERENT PARTS OF THE FROG'S STOMACH

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It has been shown in a recent paper that strips of muscle cut from different parts of the wall of the mammalian stomach vary markedly in their tendencies to rhythmic contraction, and in the forms of the curves obtained (1). Similar differences were found in the behavior of circular strips from the cardiac and pyloric regions of the frog's stomach. Marked variations were found also in the irritability and latent period when different parts of the stomach were stimulated with the same strength of current (2).

The question now arises, what is the origin of these differences? Are there peculiarities of structure and metabolism behind them, and if so, are they in the muscle, in the nerves, or in both? The fact that the strips would often beat better on the second or third day after excision than on the first, and that they would show some rhythmicity even after five days, made it hard for me to accept Magnus' view that the rhythm is dependent upon Auerbach's plexus (3). Although the myenteric nerve cells are peculiarly resistant (4), one can hardly imagine their functional capacity increasing forty-eight to seventy-two hours after death. It seems probable enough that the nerve net regulates and profoundly influences the muscle, but recent work on the cultivation of tissues has shown conclusively that it is not essential to the institution of rhythmic contraction. Muscle cells will beat in the test tube when there can be no question as to the absence of nervous tissue (5). Recently Gunn and Underhill (6) have repeated Magnus' work, and have obtained rhythmic contractions in strips from which it would seem certain that all remnants of the plexus had been removed. Their pharmacological studies, using nerve-ending and

muscle stimulants at varying intervals after the removal of the strips showed that the muscle lost its rhythmicity when it seemed to die, that is, when it no longer reacted even to barium chloride. This was usually after four or five days. It would seem best, then, to look for peculiarities in the muscle itself, peculiarities that would explain the different behavior of the strips from the different regions.

The rate of rhythmic contraction seems to be suited to the metabolism of the muscle. As Woodworth says (7), "Both activity and rest are good for a muscle. If the pause preceding a contraction is too short, the contraction suffers from lack of recuperation. If it is too long, the contraction suffers from what we may perhaps call a sort of drowsiness." If we speed up the metabolism of the muscle by warming it, the rate of rhythmic contraction is increased; and with artificial stimuli, the latent period is shortened, and the form of the curve greatly changed. These changes are well shown by Stewart (8). They probably account for De Zilwás (9) finding that, with the muscle at 40°C., stimuli had to follow each other within from five to ten seconds if summation was to be obtained. At 25°C. the interval could be twenty seconds or more. Similarly, Engelmann (10), while studying the ureter of the rabbit, found that a series of subliminal stimuli might bring about a contraction if the interval between them was not too great for summation. When the irritability was high and the waves were traveling rapidly down the ureter, the interval had to be shorter. It had to be shorter also when using the ureter of the rat, as there the rate of the waves is normally faster than in the rabbit. Woodworth (11) found that the smallest interval at which two stimuli can come, so that the effect of each shall show separately on the curve, varies widely in stomach strips from different frogs. It seems to me, from all this, that when we find, in the same animal, a strip of duodenum contracting twenty times a minute and a strip of ileum contracting ten times, we should expect to find a more active metabolism in the one than in the other. The muscle in the duodenum may be different from that in the ileum, much as the pectoral muscles of a bird are different from the thigh muscles.

Ranvier (12), years ago, commented upon the anatomical differences in smooth muscle from the aorta, from the arterioles, intestine, bladder, uterus, etc. In the arteries and in the intestine the fibers are generally quite parallel; in the bladder of the frog there is a marked reticulum, formed by the interlacing of tiny bundles of muscle. McGill (13) has shown that there persists in various parts of the digestive

tract an embryonic type of smooth muscle which can be recognized by its branching fibers and syncytium formation. Grützner (14) warns us not to take strips from the region of the pylorus in the frog, because he says the fibers are not parallel there but interlace diagonally. He says it is well known that different examples of smooth muscles (from different animals and different organs) have different irritabilities, different latent periods, and forms of the contraction curve. See table on page 56 of his article.

Pompilian, also, (15) found big differences in latent period and duration of contraction between the smooth muscles of the eye-tentacles and those of the foot of the snail. To the naked eye, the tentacle muscle is paler than that of the foot. He could not make out histological differences, but he felt they must be there. Lapicque (16) showed that the duration of stimulus necessary to bring about a contraction varies markedly in different examples of muscle from lower animals. I can find no reference to differences in muscle in different parts of the digestive tube. The nearest approach to it is the opinion expressed by some writers that the irregular contractions met with were due to an interference of waves originating in fibers of different rhythmicity (17). Thus, in the esophagus of the toad, Bottazzi (18) found that the longitudinal fibers contract more frequently than do those of the circular muscle.

It must not be forgotten that there may be physiological differences also in the nerves associated with these muscles. Local anatomical differences in the nerve net have been commented upon in previous papers (19). Carlson's work (20) suggests that more irritable and rapidly conducting nerves will probably be found in connection with the quicker muscles.

It was this literature which encouraged me to look for differences underlying the big differences in rhythmicity which I have found in various parts of the digestive tract. I wished to see also if the muscle strips from different regions would retain those peculiarities of irritability, latent period and form of contraction observed in the intact stomach, and described in the last paper. Work was done first on the stomach of the frog on account of its simplicity. Most of the writers on smooth muscle have used circular strips from the frog's stomach, but I can find no reference to differences in the behavior of strips from different parts of the organ. The middle strip has generally been used. Schultz (21) says he used pieces from a little below the middle, and his tracings show that they must have come from the upper

antrum. It should be explained here that the pyloric end of the frog's stomach is different from the cardiac end. It is firmer to the touch; the wall is thicker, and its outer surface is smooth and glistening.

TECHNIC

Narrow strips were obtained from the cardia, the middle region, and the pyloric antrum. Several difficulties had to be overcome before a satisfactory technic could be worked out. Since differences have been observed in the behavior of strips with and without the mucosa (22), it seemed best to preserve it carefully at all times so that the conditions would be more comparable. No difficulty was encountered in cutting strips from the cardiac region, as the mucous membrane is there closely adherent. Near the pylorus, however, the greatest care had to be taken or the mucosa would slip off and be lost. This laxity of the submucosa in the antrum pylori was marked in the higher animals also. Most of the rings were cut with a razor, and then carefully opened out. They were from 3 to 5 mm. wide. Those from the cardiac end were longest, but this disparity in length could apparently be disregarded, as the cardiac strips reacted the same, before and after being trimmed to the length of the pyloric ones.

These strips were suspended in a moist chamber similar to that described by Porter (23). It was made from a six ounce specimen bottle lined with blotting paper. This bottle was kept in a water bath at 19°C. The light heart lever was weighted with 1 gram; just enough to balance the preparation and to keep it taut. The faradic current was used. It was obtained from a Harvard Apparatus Company's inductorium (24) with one dry cell, and was led to the two little serrefines grasping the ends of the strips. Each strip was stimulated first with the secondary coil at 6 cm. and later with it at 4 cm. No reactions could be obtained with the coil at 8 cm. The duration of the stimulus was about one second; longer with the weaker current and more sluggish strips, shorter with the stronger current and more irritable strips. Such slight differences in stimulation did not seem to have any effect on the latent period or upon the form of the contraction curve. Some work was done with strong make and break induction shocks. Only the break seemed to have any effect, and this was much less marked than that of the tetanizing current. The latent period was longer with the single shock, but otherwise the shapes of the contraction curves were the same. Five minutes were generally allowed between each

stimulus for the recovery of the muscle. It was found later that this interval might have to be longer when studying the strips from the cardia, and that it might be much shorter for the antral strips. California frogs were used, rana draytonii and rana temporaria. As was to be expected, there were marked individual differences, due probably to infection with parasites, condition of the reproductive organs, etc. The poorest strips came from some animals that were left over night in a steam-heated room. Most of the work was done between October and January. Better results were obtained in six frogs studied about May 1. Their stomachs generally contained food, and they were more active than were the winter frogs.

LATENT PERIOD

Figure 1 represents the latent periods obtained in studying strips from the two ends of the stomach in nineteen frogs. The lines connect successive readings on the same strip. The first and third columns represent figures from the cardia; the second and fourth represent those from the antrum. The first two columns of figures were obtained with the secondary coil at 6 cm.; the next two were obtained with the coil at 4 cm. The strips were always stimulated with the weaker current first, as the stronger stimuli often seemed to injure the muscle.

It will be seen at a glance that the latent period was longer and more variable in the cardia than in the antrum, and this was true for both strengths of current. The widest variations were seen in the cardiac strips when stimulated with the coil at 6 cm. A number of factors may have been active in bringing out these differences. In the first place, the cardia seemed to suffer much more from the trauma of excision than did the antrum. Recovery from this trauma was seen in the progressive shortening of the cardiac latent periods for some time after attachment. Typical examples of this are shown in the following table. The figures represent seconds.

TABLE 1

∞ ; 4.2; 3.4; 2.7; 1.4; spontaneous contractions; 4.6
∞ ; 2.2; 2.3; 1.5
∞ ; 8.0; 4.0

The first experiment is particularly instructive, as the strip which at first failed to react at all, after 25 minutes, reacted in 1.4 seconds. The latent period of the corresponding strip from the antrum was, in

successive tests, 4.8, 4.3, and 4.0 seconds. Here, again, improvement is to be noted but it is not so marked as in the cardiac strip. In a number of instances, the three or four successive tests on an antral strip gave the same figure for the latent period. This is shown by

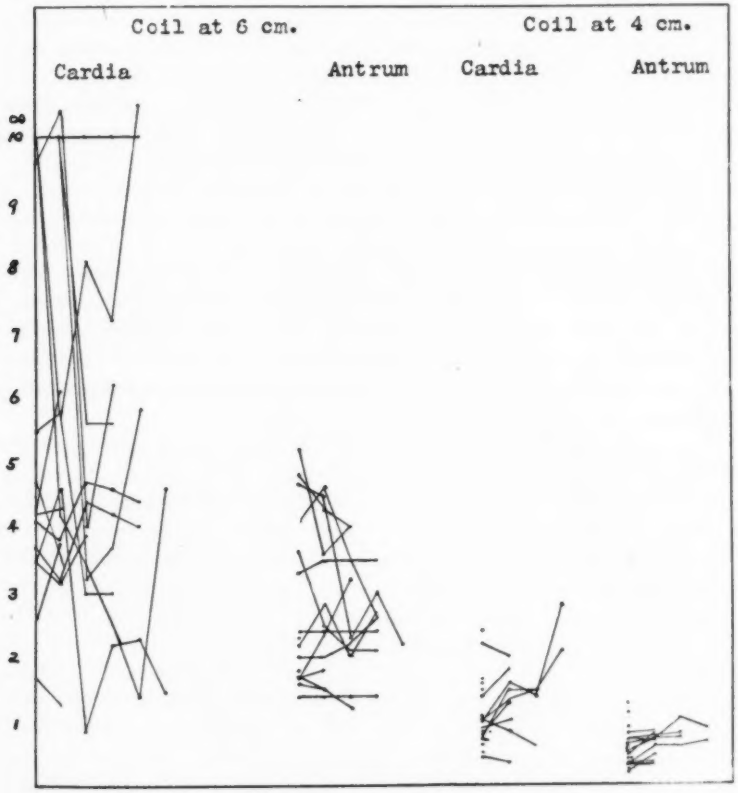

Fig. 1. Latent periods of cardiac and pyloric muscle strips from the stomachs of nineteen frogs. Ordinates represent seconds; the lines connect successive tests on the same strip.

the number of horizontal or nearly horizontal lines in the second and fourth columns of figure 1. I have commented elsewhere on a similar difference in vulnerability between segments from the duodenum and from the ileum (25). When a number of such segments are put into

warm oxygenated Ringer's solution, those from the ileum will generally begin beating first, and they will stand much more trauma than will the more sensitive duodenal segments.

The great rhythmicity of the cardiac strips interfered considerably with the work. In the first place, it made the records difficult to read on account of the changing base line, and at times it was hard to say whether or not a rise after stimulation was wholly or in part spontaneous. A certain refractoriness often appeared with the onset of rhythmic contractions. This did not correspond to that observed with heart muscle, as a contraction could easily be superimposed upon any part of a spontaneous wave, but there was a lowering of irritability and a lengthening of the latent period. Returning to the table, it will be seen in the first protocol that the appearance of rhythmic movements was followed by a lengthening of the latent period from 1.4 to 4.6 seconds. Sudden alterations in irritability often gave me the impression that rhythmic changes were going on in the muscle although there were no visible contractions. These suspicions have been strengthened by Stübel's (26) observation that rhythmic action-currents can be obtained from the stomachs of birds even after the contractions have been inhibited.

A number of methods were tried in the hope of stopping the rhythmic waves without interfering with the irritability of the strips. Schultz's (27) method of removing the mucous membrane and soaking in atropin solution was tried on a number of frogs, but it seemed to injure the cardiac strips more than it did the antral ones, so it could not be used. Sometimes the movements could be inhibited by a series of subliminal stimuli. The best results were secured by stimulating just after the muscle had relaxed from an artificial contraction and before the spontaneous waves could begin again. No such difficulties were encountered with the antral strips, which showed very little rhythmicity.

Not only were there these rhythmic movements to contend with, but sometimes there were slow variations in tone. Jordan (28) believes that a change in tone greatly alters the irritability of smooth muscle, and it may be that the low irritability of the cardiac strips at first was due partly to their increased tone. Grützner (p. 27) quotes the literature to show that smooth muscle when tonically contracted will not react to the strongest induced currents. He found this true for freshly cut stomach rings; but if he killed the frog, left it in a cool room for 24 hours, and then cut a ring, it was much more irritable and

reacted well. I repeated this work, and found that the improvement was to be noted only in the strips from the antrum. This subject will be taken up again later. I do not believe that changes in tone affected the reactions very much. The strips from the antrum showed the most even base line, just as they did when beating in Ringer's solution.

When we remember how much shorter the latent period was at the cardia than in the antrum of the intact stomach, it is surprising to find the conditions reversed in the strips. Excluding the negative results, the averaging of 172 readings in 21 frogs gave the following figures:

TABLE 2

Cardia	*Antrum*
6 cm. 3.90 seconds	2.70 seconds
4 cm. 1.26 seconds	0.66 seconds

In only two frogs were the lowest figures obtained with the cardiac strip less than the lowest for the antral strip from the same stomach. The protocols are as follows:

TABLE 3

Frog	*Coil Distance*	*Cardia*	*Antrum*
1	6 cm.	4.2; 3.4; 2.7; 1.4; 4.6	4.8; 4.3; 4.0
	4 cm.	1.60	1.15
2	6 cm.	3.5; 4.6; 0.9; 2.2; 2.3; 1.5	2.4; 2.4; 2.4; 2.4
	4 cm.	0.43; 0.38	0.55; 0.75

The second protocol is very definite, as the results with the two strengths of current are in accord. This evidence that the cardiac strips can at times react more quickly than the antral ones raises the question as to whether they might not always do so if it were not for the greater sensitiveness to trauma, the greater rhythmicity and refractoriness. It will be seen later that, in the mammalian stomach, the latent period of the strip from the cardiac region was usually shorter than that of the antral strip. If, in the frog, the latent period of the *muscle* in the antrum is really much shorter than that around the cardia, it would seem that the greater irritability of the cardia in the intact stomach must be attributed to the activities of the nervous plexus. In the intact stomach, the cardia responds to stimulation with the coil at 13 cm. and the antrum with it at 8 cm. Excised strips from both regions react only when the coil is at 6 cm., hence the loss of irritability is greater proportionately in the cardiac strips. Since

Barbera's (29) results on the excised stomach agree closely with my own for the organ in situ, it would appear that the disproportionate lengthening of the latent period must be due more to the cutting of the strips than to the stoppage of the circulation and the severance of the extrinsic nerves.

DIFFERENCES IN THE REACTION TO TRAUMA

Several writers have commented upon the injurious effects of strong induced currents on smooth muscle. Apparently the cardiac strips suffered from this more than did the antral ones, judging by the degree of lengthening of the latent period after successive stimuli (with the coil at 4 cm.). Some of the antral strips did not seem to be injured at all, while a few of the cardiac ones lost their irritability entirely after one or two shocks. The following protocols of experiments with the secondary coil at 4 cm. bring this out.

TABLE 4

Cardia	*Antrum*
0.80; 1.30; ∞	0.32; 0.35
0.77; 1.50; 1.50; 2.10	0.35; 0.60; 0.60; 0.70
1.00; 1.60; 1.40; 2.80	0.72; 0.70; 1.15; 0.90
1.10; ∞	0.80; 0.85
2.80; 4.40; 6.40	1.40; 1.20; 2.10
0.85	0.75; 0.60; 0.50

This difference was most marked in diseased or weakened animals in which the cardia showed very little vitality. The impression was gained while studying the mammalian stomachs also, that the more sensitive cardiac muscle suffers most from a general lowering of the bodily strength and resistance. Perhaps for the same reason, the cardiac strips did not stand being kept in the ice box for twenty-four hours so well as did those from the antrum. After the pieces had been tested, they were put in Ringer's solution, usually at 6°C., and the reactions were taken again next day. Some kept well, and contracted with as good amplitude as they showed at first; others, usually from the weaker animals, reacted poorly. The following figures show that the latent period in the antral strips may even be shorter after twenty-four hours. This was observed quite constantly, later, with antral strips from the dog. The figures in the following table represent the shortest periods obtained during the usual series of tests on each strip.

TABLE 5

FROG	COIL DISTANCE	CARDIA			ANTRUM		
		After excision	After 24 hours	Per cent	After excision	After 24 hours	Per cent
	cm.						
1	6	2.60	3.20	123	2.30	1.70	73
	4	0.85	1.75	205	0.65	0.61	93
2	6	3.50	∞	∞	1.70	3.00	176
	4	0.65	2.20	338	0.40	0.30	75
3	4	2.00	3.90	195	1.30	1.80	138
4	4	1.50	3.40	226	0.55	0.85	154

After the 24 hour interval, the amplitude was much better in the antral strips than in the cardiac ones.

STRIPS FROM THE MIDDLE OF THE STOMACH

In eight frogs, a strip was cut from the middle of the stomach. All but one of these resembled in their reactions the corresponding cardiac strips, although the latent periods were sometimes shorter. As the frog's stomach has no distinct upper limit, its mid-point could not always be located with accuracy, and it may be that some of the strips were taken, really, from the upper edge of the pyloric antrum. These strips from the middle stretched out a great deal and did not seem to have much tone.

SHAPE OF THE CONTRACTION CURVE

A very definite difference was found between the type of curve traced by the cardia and that of the antrum (see fig. 2). The following table shows latent period, apex and relaxation times expressed in seconds elapsed after stimulation.

The better amplitude usually met with in the antral strips made it easier to get good tracings from them than from the cardiac ones. I have remarked elsewhere on the wide amplitude of the *spontaneous* contractions in the antrum. The relaxation time, particularly in the cardiac strips, seemed often to be shortened or lengthened according as the tone happened to be falling or rising. When it was rising, the lever generally did not return to its old level at all. After twenty-four hours, the relaxation time was sometimes shorter, sometimes longer. The slower relaxation of the cardiac strips may be due to the greater sensi-

TABLE 6

COIL DISTANCE	CARDIA			ANTRUM		
	Latent period	Apex	Relaxation	Latent period	Apex	Relaxation
cm.						
6	1.30	32	480	0.95	20	160
	3.20	29	265	1.90	16	50
	3.50	67	217	0.55	21	89
4	0.50	22	290	0.48	16	150
	0.85	30	480	0.20	13	190
	1.75	45	400	0.65	18	164
				0.61	18	114
				0.85	21	90
				0.40	17	206
				0.35	12	70
Averages.....	1.85	37	355	0.69	17	130

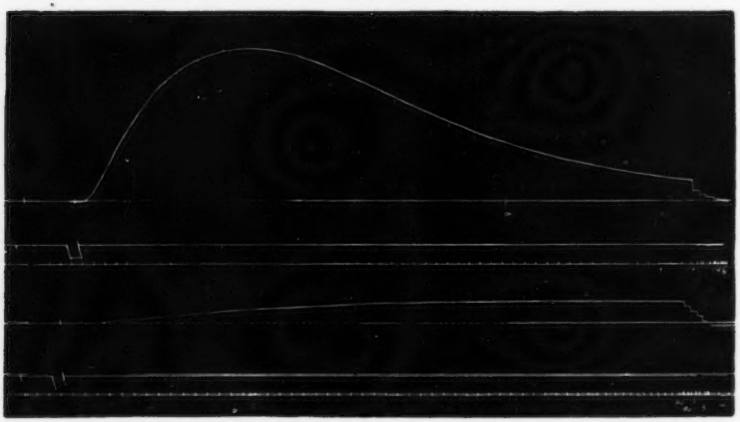

Fig. 2. Contraction curves from strips from the antrum (upper) and the cardia (lower). The same strength of faradic current was used. Time markings represent seconds. Magnification of lever 4.5:21.

	LATENT PERIOD	APEX	RELAXATION
Antrum.....	0.6	16	145
Cardia.....	2.2	45	200

tiveness to trauma or it may possibly be a useful reaction, suited to the functions of that region which are to keep the upper part of the stomach closed, and to press the gastric contents steadily into the antral mill.

It was rather surprising to find that the latent period of smooth muscle from the antrum of the frog's stomach was often shorter than that of muscle from the antrum of the rabbit, cat and dog (the stimulus, of course, being the same). This is the more surprising in that the frog strips were tested at 19°C., the others at 37°C. Another surprise was that the frog strips did not keep so well in the ice box (in cold blooded Ringer's solution) as did those from the dog.

SUMMARY

Marked differences have been found in the reactions of muscle strips from different parts of the stomach of the frog, stimulated in a moist chamber with the faradic current.

The cardiac strips showed by far the greatest rhythmicity. They seemed to suffer most from the trauma of excision, and they recovered slowly as compared with the antral strips. They suffered more damage, also, from strong faradic currents than did the antral strips. They did not stand being kept in the ice box for twenty-four hours so well as did the strips from the antrum. The latent period of the antral strips at times was even shorter after such treatment.

The latent period almost always was longer and more variable in the cardiac than in the antral strips. In only two out of twenty-one frogs did the cardiac muscle respond more quickly than the antral.

Strips from the middle of the stomach generally resembled the cardiac ones in their behavior.

The shape of the contraction curve was different for cardiac and antral strips. The contraction and relaxation times were shorter, and the amplitude was greater in the antral strips.

BIBLIOGRAPHY

(1) Alvarez: This Journal, 1916, xl, 585.
(2) Alvarez: This Journal, 1916, xli, 321.
(3) Magnus: Arch. f. d. gesammt. Physiol., 1904, cii, 361.
(4) Cannon and Burket: This Journal, 1913, xxxii, 347; Scandola: quoted from Journ. Amer. Med. Assoc., 1915, lxiv, 2029.

(5) Burrows: Münch. med. Wochenschr., 1912, xxvii, 1473; Lewis: This Journal, 1915, xxxviii, 153; see also Verzar: Arch. f. d. gesammt. Physiol., 1914, clviii, 419, and Lake: Journ. Physiol., 1916, l, 364.
(6) Gunn and Underhill: Quart. Journ. Physiol., 1914, viii, 275.
(7) Woodworth: This Journal, 1900, iii, 32.
(8) Stewart: This Journal, 1901, iv, 202, 203, fig. 14.
(9) De Zilwa: Journ. Physiol., 1901, xxvii, 216.
(10) Engelmann: Arch. f. d. gesammt. Physiol., 1870, iii, 283.
(11) Woodworth: This Journal, 1900, iii, 41.
(12) Ranvier: Lecons d'Anat. Gén., Paris, 1880, 433.
(13) McGill: Amer. Journ. Anat., 1909, ix, 508, 523.
(14) Grützner: Ergebn. d. Physiol., 1904, iii2, 32.
(15) Pompilian: C. R. Soc. Biol., 1899, li, 489.
(16) Lapicque: C. R. Acad. Sc. Paris, 1905, cxl, 801.
(17) Woodworth: This Journal, 1900, iii, 36; Stewart: Ibid., 1901, iv, 190.
(18) Bottazzi: Quoted from Grützner, Loc. cit., 59.
(19) Alvarez: This Journal, 1914, xxxv, 191.
(20) Carlson: This Journal, 1905, xv, 136.
(21) Schultz: Arch. f. Anat. u. Physiol., physiol. Abth., 1903, 1.
(22) Kautsch: Arch. f. d. gesammt. Physiol., 1907, cxvii, 113.
(23) Porter: This Journal, 1904, x, xliii.
(24) Porter: This Journal, 1902, viii, xxxv.
(25) Alvarez: This Journal, 1914, xxxv, 179.
(26) Stübel: Arch. f. d. gesammt. Physiol., 1912, cxliii, 383.
(27) Schultz: Arch. f. Anat. u. Physiol., physiol. Abth., 1903, 1.
(28) Jordan: Arch. f. d. gesammt. Physiol., 1905, cxl, 533.
(29) Barbera: Zeitshr. f. Biol., 1898, xxxvi, 239.

VII. DIFFERENCES IN LATENT PERIOD AND FORM OF THE CONTRACTION CURVE IN MUSCLE STRIPS FROM DIFFERENT PARTS OF THE MAMMALIAN STOMACH

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It has been shown recently that strips of muscle cut from different parts of the wall of the stomach vary markedly in their tendencies to rhythmic contraction and in the forms of the curves obtained (1). Decided differences were found also in the irritability and latent period when different parts of the stomach were stimulated with the same strength of current (2). In the accompanying paper (3), I have reviewed some of the literature on smooth muscle, and have given the reasons which led me to look for more peculiarities in the muscle strips: peculiarities that might account for the differences in rhythmicity. Work done first on the frog showed marked differences in the behavior of strips from cardia and antrum. They were kept in a moist chamber, and stimulated with the faradic current. The most striking difference was found in the forms of the contraction curves from cardia and antrum. The contraction and relaxation times were shorter, and the amplitude was greater in the antral strips. Contrary to expectation, the latent period was almost always shorter in the antral strips. A number of factors were mentioned which might account for this reversal of the conditions found when the stomach was stimulated *in situ*.

This paper deals with similar differences observed in the reactions of strips from different parts of the mammalian stomach.

TECHNIC

The apparatus and method have been described in the previous paper. The mammalian strips were kept at 38°C. The strips were obtained in the same way and from the same locations as described in Communication IV. They were cut longitudinally from the two cur-

vatures. The sequence in which they were tested was changed daily, although this factor did not seem to have any influence on the results. The stomach was kept in the icebox, in a moist chamber, or in Ringer's solution at 6°C., and taken out from time to time as strips were cut and tested. The strips were all about 2 cm. long. The only ones that had the mucous membrane attached were the first two from the lesser curvature of the rabbit. As explained in Paper IV, a separation was not possible in this region.

Many experiments were done at first to make sure that the results obtained could be duplicated, and that they were not accidental. It was found that with the coil at 4 cm. a series of tests on the same strip would generally show about the same latent period. For instance, a strip from the middle of the greater curvature in a dog reacted after 0.29, 0.28, 0.27 and 0.27 seconds, in successive tests at intervals of fifteen minutes. Two hours later, another strip was cut from the same region, and it reacted after 0.27 seconds. As soon as it seemed certain that single observations could be trusted, the following technic was adopted. The strips were left ten minutes in the moist chamber, that they might warm up and recover somewhat from the trauma of attachment. They were stimulated then with the coil at 6 cm. A minute after they had regained their original lengths, they were stimulated with the coil at 4 cm. Again, after waiting for relaxation, they were stimulated with the coil at 0 cm. The contractions were not quite maximal with the coil at 4 cm. When the result with C.D. (Coil Distance) 4 cm. was shorter than that with C.D. 6 cm., and a little longer than that obtained with C.D. 0 cm., it was pretty sure to be correct. Most of the discrepancies met with were due probably to the difficulties in marking the record when the lever had risen very gradually at first. The mammalian strips have been much more satisfactory to work with, however, than were the frog strips; the base line has been more constant, and in most of the records the latent period could be measured accurately. The great rhythmicity which was so troublesome while studying the frog's cardia was seldom met with while working with the rabbits' stomachs, and then only with the cardiac strip. It was more disturbing in some strips from the stomachs of cats and dogs.

LATENT PERIOD

Figures 1 and 2 represent the latent periods obtained in strips from different regions of the rabbit's stomach with the secondary coil at

6 cm. and at 4 cm. The ordinates represent fractions of seconds. The lines connect readings from the several strips from one stomach. The little diagrams above show the locations from which the different strips were taken. When a number of tests were made on the same strip, the shortest latent period observed was taken for these charts. It will be seen that with the weaker current, the shortest latent periods were observed in the two strips from the cardia and from the fundus. Many strips failed to respond at all. This was true particularly of the strips from the antrum and from the middle and preantral regions of the greater curvature. The cardiac strip also failed to react in a number of animals. The fundus showed the smallest percentage of failures, while the strip from the antrum on the greater curvature responded but once, and then doubtfully.

With the stronger current, figure 2, reactions were always obtained in normal animals. Again, it will be seen that the latent period was shortest at the cardia and at the fundus. After excluding a few widely di-

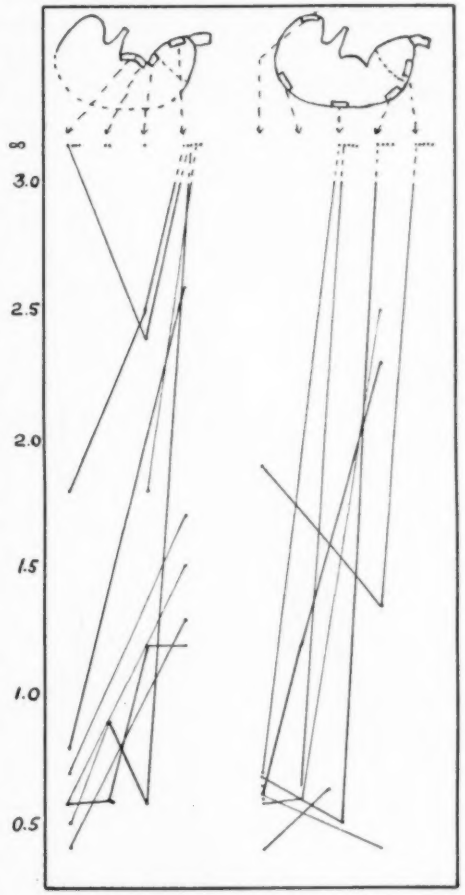

Fig. 1. Latent periods of muscle strips from the rabbit's stomach with the coil at 6 cm. The diagrams indicate the regions from which the different strips were taken. Ordinates represent seconds. The lines connect readings on strips from the same stomach.

vergent results and those obtained in animals which were found to be diseased, the averages (for the rabbit) were as follows:

Lesser curvature	seconds	*Greater curvature*	seconds
Near cardia	0.22	Fundus	0.25
Middle	0.37	Opposite cardia	0.35
Preantrum	0.35	Middle	0.40
Antrum	0.65	Preantrum	0.41
		Antrum	0.76

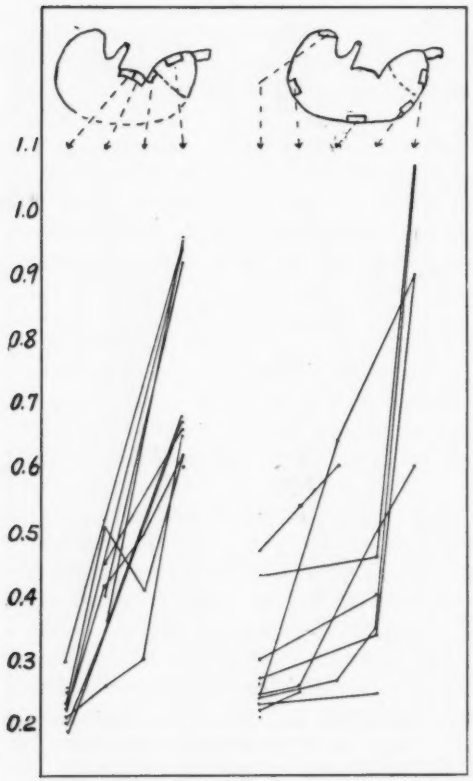

Fig. 2. This corresponds in every way to figure 1, except that the strips were stimulated this time with the coil at 4 cm.

From this we can say that, roughly, the latent period of the strips varied inversely as their distance from the cardiac end of the stomach. The latent periods of the antral strips were three times as long as those of strips from cardia and fundus.

Similar results were obtained with strips from the stomachs of a few dogs. Figure 3 shows the gradation in the latent periods of strips from different regions. Again, it will be seen that the strip from the lesser curvature next to the cardia responded usually in the shortest time, and that there is more or less gradation towards the antrum. Sometimes the latent period was shorter in the strip next to the pylorus than in the one just above in the antrum. This is in agree-

ment with the finding in the intact stomach that the pyloric ring is more irritable than any other part of the antrum.

Observations on strips from the stomachs of a few cats indicate that the latent periods are very similar to those in the dog.

A little work was done with circular stomach strips in dogs and rabbits, but it was not satisfactory on account of the poor amplitude of the contractions obtained. This made it hard to say just where the lever began to rise. As nearly as could be determined, there was a gradation from cardia to pylorus similar to that found with the longitudinal strips.

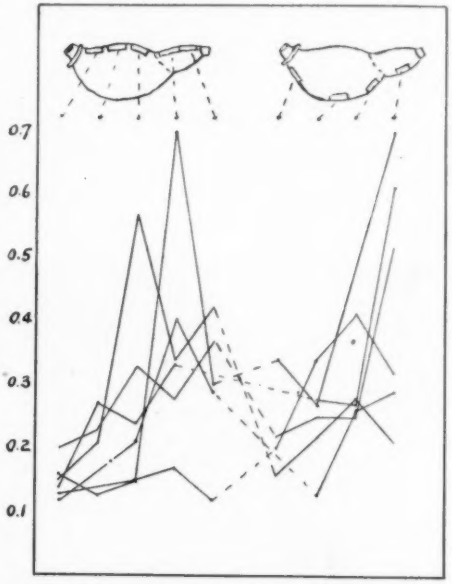

Fig. 3. Latent periods of muscle strips from the dog's stomach with the coil at 4 cm. The diagrams indicate the regions from which the different strips were taken. Ordinates represent fractions of seconds. The lines connect readings on strips from the same stomach. The lowest line on the left was from a dog that had recently been inoculated against distemper with a live culture of B. bronchisepticus. As far as could be ascertained, the other dogs were in good condition.

THRESHOLD OF IRRITABILITY

In the rabbit, the threshold was usually about the same for the four strips on the lesser curvature and for the upper two on the greater curvature. They all reacted with the coil at 6 cm. In a few animals, the cardiac and fundus strips reacted with the coil at 8 cm. In a few dogs, the strip from the lesser curvature near the cardia responded well with the coil at 10 cm., while the other strips responded only at 7 cm. and 6 cm. This was to be expected from the results on the intact stomach.

SHAPE OF THE CONTRACTION CURVE

Here, again, differences have been found similar to those already described for the frog. They perhaps can best be illustrated by the following data from the rabbit. The figures indicate seconds elapsed after stimulation. The coil was at 4 cm. Sample tracings are shown in figure 4.

	LATENT PERIOD	APEX	RELAXATION
		Cardia, lesser curvature	
			seconds
	0.20	5.6	30
	0.30	5.6	31
	0.23	5.0	17
	0.25	5.0	40
	0.25	5.0	45
	0.25	6.4	45
	0.21	5.2	35
Average...	0.24	5.4	35
		Fundus, greater curvature	
	0.23	13.0	After 180 seconds it had relaxed one-third.
	0.21	12.4	After 120 seconds it had relaxed very little.
	0.47	10.6	After 120 seconds it had relaxed very little.
	0.34	17.0	After 14 minutes it had relaxed very little.
	0.37	56.0	After 10 minutes relaxation was not complete.
	0.43	15.0	After 10 minutes relaxation was not complete.
	0.27	30.0	After 10 minutes it had relaxed very little.
	0.24	13.0	After 5 minutes it had relaxed very little.
	0.24	12.0	After 120 seconds it had relaxed very little.
Average...	0.31	20.0	?
		Antrum, lesser curvature	
			seconds
	0.68	5.2	15
	0.95	4.7	18
	1.00	4.6	14
	0.95	4.2	16
	1.50	4.3	19
	0.65	4.0	17
Average...	0.95	4.5	16

	LATENT PERIOD	APEX	RELAXATION
		Preantrum, greater curvature	
	0.25	7.4	After 50 seconds it had relaxed two-thirds.
	0.46	6.3	After 60 seconds it had relaxed completely.
	0.34	6.0	After 31 seconds it had relaxed completely.
	0.36	5.5	After 27 seconds it had relaxed completely.

Enough data have not been secured from other parts of the stomach to give averages of any value. The figures from cardia, fundus and antrum show clearly enough that there were differences in the shape of the curve sufficiently characteristic, if need had been, to have identified the different strips.

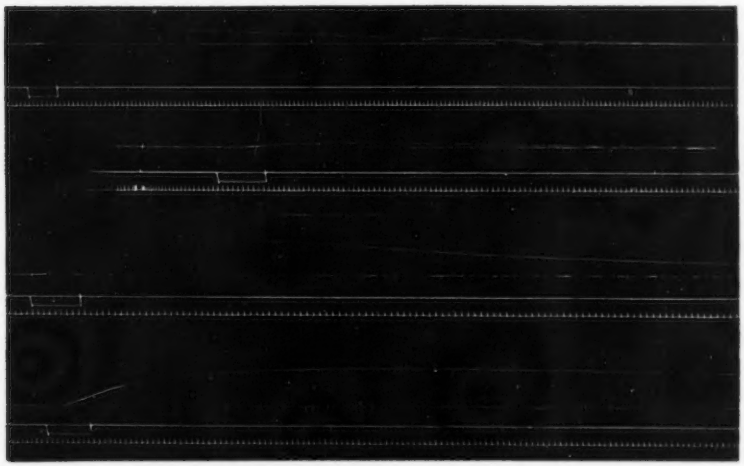

Fig. 4. Sample tracings from four strips from the rabbit's stomach stimulated with the coil at 4 cm. From above downward, they are from (*1*) the lesser curvature near the cardia; (*2*) antrum on the lesser curvature; (*3*) greater curvature just above the antrum, and (*4*) top of fundus. The magnification was the same for all. The time record represents seconds.

The antrum had the shortest contraction and relaxation times of any part of the stomach. It is strange that, with these, it should have had the longest latent period. One can not help thinking of the work of Lee, Guenther and Meleney (4) who showed that while the diaphragm excels the other skeletal muscles in almost every way, it falls

behind them as regards its latent period. I have already suggested (2) that the muscle in the antrum may be different from that in the rest of the stomach, just as the diaphragm is different from other muscles, and for the same reason: because it works so constantly day and night.

The sustained contraction of the muscle from the fundus may be suited to its function. I have commented elsewhere on the fact that when strips from the fundus were put into warm aerated Ringer's solution, they gradually contracted down until they lost all tendency to rhythmic movement. This may have been due to a large contraction remainder.

The amplitude varied in the different strips. This can be seen in figure 4. In the rabbit, the antrum generally had the smallest amplitude, the fundus the greatest. The figures in the following table were obtained while using the same strength of current and the same lever magnification—4.5: 20.5. As stated before, the strips were cut with as nearly as possible the same length. The distance is given in millimeters from the base line to the highest point on the curve.

RABBIT	CARDIA	ANTRUM	FUNDUS
1	2.0	1.0	5.0
2	4.0	1.0	37.0
3	2.5	1.0	42.0
4	2.0	1.0	8.0
5	4.0	1.0	7.0
6	4.0	1.5	22.0
7	8.5	1.5	30.0

The figures for the fundus would have been even larger if the strips had not already, as a rule, been shortened by previous stimulation. It is hard to say why the amplitude was so poor in the antral strips. It was very good in the frog's antrum.

Differences were found in the shape of the curves also while studying the stomachs of dogs and cats. On the whole, the curves from the dog's stomach resembled those from the corresponding regions in the rabbit, but there were wider individual variations. Even the same strip would at times show strange differences, due possibly to changes in tone. For instance, a strip from the greater curvature reacted as follows:

LATENT PERIOD	APEX	RELAXATION
seconds	*seconds*	*seconds*
0.29	10	35
AFTER A FEW MINUTES		
0.28	38	500

Sometimes the shorter curve would follow the longer. A rapid return of the lever to the original base line might at times be due to a progressive fall in tone; occasionally it seemed due to the low amplitude, that is, the lever did not have far to fall.

Strips from the antrum of the dog generally showed a bigger amplitude than did those from the rabbit.

EXPERIMENTS ON THE HUMAN STOMACH

Through the kindness of Dr. L. L. Stanley, I was able to get strips from a normal human stomach at an autopsy held half an hour after death by hanging. Death was instantaneous; the man was middle aged, powerfully built and healthy. His stomach was full of food. The strips were cut about 10.30 a.m., strung on a small safety pin in known order, and dropped into Ringer's solution previously cooled to about 6°C. The laboratory could not be reached until 7 p.m., when the temperature in the thermos bottle was 13°C. The tests were made between 7.30 and 11 p.m.

The differences in latent period will be seen in the following table. The figures indicate seconds.

COIL DISTANCE	LESSER CURVATURE			GREATER CURVATURE		
	Cardia	Preantrum	Antrum	Fundus	Middle	Preantrum
cm.						
8	2.90	∞	∞	∞	∞	∞
6	0.44	0.68	0.70	0.45	0.55	0.85
4	0.19	0.27	0.28	0.22	0.32	0.26

These figures show a gradation from cardia to pylorus similar to that found in the rabbit, dog and cat. It is interesting, also, that the threshold was lowest in the cardiac strip. This strip was also the only one which showed enough rhythmicity to interfere with making the

tests. The peculiar characteristics of the cardiac strip might have been even more pronounced if, in this case, it had not been taken from a point considerably to one side of the median line. This was done in order that the area heretofore found so interesting might be studied histologically under ideal conditions.

The following figures show the differences in the shape of the curves from different regions. They denote seconds elapsed after stimulation. Relaxation was often so slow after a certain point that I could not afford to wait for its completion.

COIL DISTANCE	LATENT PERIOD	APEX	RELAXATION
		Cardia, lesser curvature	
cm.		*f*	
6	0.62	12.3	After 100 seconds little change took place.
4	0.19	25.0	After 100 seconds little change took place. Relaxation was two-thirds accomplished.
		Antrum, lesser curvature	
6	0.85	5.7	After 20 seconds relaxation was complete.
4	0.28	11.0	After 30 seconds relaxation nearly complete. It was complete in 60 seconds.
		Fundus, greater curvature	
6	0.45	6.5	After 60 seconds relaxation was complete.
4	0.22	6.6	After 300 seconds relaxation was two-thirds accomplished.
		Middle, greater curvature	
6	0.60	8.3	After 22 seconds relaxation was complete.
4	0.32	10.0	After 300 seconds relaxation was five-sixths accomplished.
		Preantrum, greater curvature	
6	0.85	9.3	?
4	0.26	60.0	After 300 seconds relaxation was six-sevenths accomplished.

No conclusions can be drawn from single experiments. It is interesting, however, to note that the antral strip relaxed promptly, as it did in the rabbit and dog.

THE LATENT PERIOD AFTER FORTY-EIGHT HOURS

While studying strips from the frog's stomach, it was found that those from the cardia did not keep well in the icebox, and their latent periods always were lengthened. Those from the antrum, however, would sometimes have a shorter latent period after twenty-four hours than they did at first. This peculiarity in the behavior of strips from the pyloric region was even more marked in the dog, where it could be observed after forty-eight hours. Between tests, the strips were kept in Ringer-Locke's solution at 6°C. The following figures show that while the forty-eight hour interval caused the cardiac latent period to lengthen 30, 136 and even 500 per cent, that of the antral strip was usually shortened; in one animal, by 75 per cent.

	CARDIA LESSER CURVATURE		ANTRUM LESSER CURVATURE		MIDDLE, GREATER CURVATURE	
Dog 1						
C. D. 4 cm	0.16		0.70		0.27	
After 48 hours	0.27		0.18		0.60	
Percentage		168		25		222
Dog 2						
C. D. 4 cm	0.22		0.35		0.29	
After 48 hours	0.52		0.31		0.70	
Percentage		236		88		241
Dog 3						
C. D. 4 cm	0.24		0.31		0.63	
After 48 hours	0.35		0.34		1.10	
Percentage		146		110		174
Rabbit						
C. D. 4 cm	0.20		0.68		0.50	
After 24 hours	1.20		0.40		∞	
Percentage		600		58		
Cat						
C. D. 4 cm	0.30		0.85		0.31	
After 24 hours	0.39		0.30		0.32	
Percentage		130		35		103

It would seem as if the antral muscle were not only much more resistant to the conditions that bring about death, but that it must, during the 48 hours, get rid of some form of inhibition.

DIFFERENCES IN DISEASED ANIMALS

Before the work had progressed far, it was noticed that, with certain stomachs, all but one or two of the strips would react poorly, the

irritability would be low, the latent period longer than usual, and the amplitude small. These strips did not keep well in the icebox, and after twenty-four hours, might not react at all to strong stimuli. The following few figures from the rabbit show that, compared with the averages for healthy animals, the strips from the fundus and middle of the greater curvature did not suffer at all. Those from the lesser curvature, however, suffered a good deal.

Rabbit

	LESSER CURVATURE			GREATER CURVATURE				
	Cardia	Prean-trum	Antrum	Fundus	Opp. Cardia	Middle	Prean-trum	Antrum
March 15, 1915.....	0.85		1.00	0.21		∞		
December 6, 1915.....	0.60	0.90	1.40	0.22	0.25	0.50		0.60
April 3, 1916.....	1.05		1.50	0.27				
July 8, 1916.....	0.70	1.15	1.50		1.43	0.27	1.65	∞
Normal average.....	0.22	0.35	0.65	0.25	0.35	0.40	0.41	0.76

C. D. was 4 cm. in all.

The first animal seemed to have some intestinal disturbance as the bowel was distended and flabby; the second had snuffles; the third had large, inflamed Peyer's patches and some indurated areas in the cecum, due apparently to encysted parasites; while the fourth had recently had an abortion, judging from the appearance of the uterus.

Similar deviations from normal were found in strips from the stomachs of distempered dogs. There, the most marked feature was the great shortening of the latent period in the strips from the pyloric antrum. In two dogs, this shortening caused an actual reversal of the gradient, the antral strip contracting before the cardiac one. The figures obtained on successive tests with the coil at 6 cm. (4 cm. unfortunately was not used) are as follows:

Lesser curvature	*Lesser curvature*
Cardia	*Antrum*
Small male spitz.	0.40; 0.45; 0.40; 0.39	0.22; 0.30; 0.30
Mongrel female.....	0.85; 0.90; 0.90	0.65; 0.60; 0.65; 0.70; 0.70

It will be noted, in figure 3, that one of the lines is unusually low, and that the antrum responded more promptly than the cardia. These records were included in the chart because the dog seemed healthy and normal in every way. Inquiry showed later, however, that that dog

had recently been inoculated against distemper with a live culture of *Bacillus bronchisepticus*. It seems probable, then, that the reversal of the gradient on the lesser curvature was due to this infection.

The following figures are from cardia and antrum of a large old mongrel dog which had been vomiting for several days. He was *in extremis* when killed by ether and bleeding. Autopsy showed a severe chronic nephritis. One kidney contained a stone, and the prostate was markedly hypertrophied. The lungs were normal.

Lesser curvature	*Lesser curvature*
Cardia	*Antrum*
C. D. 8 cm. ∞	∞
C. D. 6 cm. 0.57	∞
C. D. 4 cm. 0.19	0.18
C. D. 0 cm. 0.22	0.17

It will be seen that although the irritability was greater in the cardiac strip, the latent periods with strong stimuli were shorter in the antrum. The reversal of the gradient was due mainly to the marked shortening of the antral latent period. A similar reversal was found in the stomachs of two monkeys killed in the last stages of dysentery.

DISCUSSION

The gradation in the latent period from cardia to pylorus on both curvatures agrees with the gradation of rhythmicity previously described. This work has emphasized again the peculiar properties of the muscle in the cardiac region on the lesser curvature, and has made it seem more likely that that is a rhythmic center for the stomach. There was some question at first whether or not there might be another such center in the preantral region on the greater curvature. The waves often seem to arise there, and strips from that area generally showed considerable rhythmicity. This work indicates now that the muscle there is not very different from that in the areas near by. Certainly it does not stand out as peculiarly as the cardiac muscle does.

It is possible that the greater irritability and shorter latent period in the lower than in the upper part of the antrum might tend to stop the waves as they reach the pylorus. It is well known that the waves do not sweep over into the duodenum. Often in the rabbit I have seen, much as Auer (5) has described, a small contraction appear at the pylorus and, moving upwards, break against the down-coming

wave in the lower antrum. The process makes one think of backfiring in a forest. This might occur, perhaps, if the shorter latent period in the muscle of the pyloric ring should cause it to pick up the stimulus ahead of its turn.

If the gradation of latent period, irritability, rhythmicity, etc., from cardia to antrum plays any part in the normal progress of peristalsis, it seems likely that an upset in this gradient should have important consequences. There might then be a tendency to anastalsis, the stomach would not invite food, and appetite might be lost. It must be remembered, of course, that the figures given here have been obtained from excised strips, stimulated powerfully. The usual stimuli received by the stomach are probably much weaker. Moreover, the gradient of latent period and irritability for such stimuli in the highly sensitive intact stomach is probably steeper than it is in the strips. Even in the strips, the gradient is generally steeper with weak stimuli. Thus in the table of results on the human stomach, it will be seen that with the coil at 8 cm. the gradient is from 2.90 seconds at the cardia to ∞ at the pylorus; with the coil at 4 cm. it is from 0.19 second at the cardia to 0.28 second at the pylorus. We can not say, as yet, how much the gradient can be changed in the intact stomach. Certainly it is interesting to know that the muscles in cardia and antrum are so different that the one can be slowed and the other quickened by the same agent. The subject invites further research.

SUMMARY

Differences have been found in the latent period of muscle strips removed from different parts of the stomach wall. Speaking roughly, the latent period varies inversely as the distance from the cardia.

The irritability was greatest in the strip from the lesser curvature next to the cardia.

There were differences in the shape of the contraction curve sufficiently characteristic, if need had been, to have identified a number of the different strips.

Similar results were obtained while studying strips from a human stomach.

When the strips were left 48 hours in the icebox, those from the antrum showed themselves peculiar in that their latent periods were shorter, while those of the other strips were more or less lengthened.

In diseased animals, all but one or two strips generally showed a

lessened irritability and a lengthened latent period. In some animals, the latent period of the antral strip was markedly shortened. The lengthening at the cardia and the shortening at the pylorus upset the normal gradient in several animals. It seems likely that if such changes should take place in the intact stomach, digestion might be embarrassed.

BIBLIOGRAPHY

(1) Alvarez: This Journal, 1916, xl, 585.
(2) Alvarez: This Journal, 1916, xli, 321.
(3) Alvarez: This Journal, 1917, xlii, 422.
(4) Lee, Guenther and Meleney: This Journal, 1916, xl, 446.
(5) Auer: This Journal, 1908, xxiii, 165.

REFLEX RESPIRATORY CHANGES AND THE CONCOMITANT THRESHOLD STIMULUS

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Considerable work has been done on the ways in which respiration may be modified reflexly by stimulating cutaneous sensory nerves (1), e.g., sciatic, radial, ulnar, median and trigeminal, or by stimulating visceral sensory nerves (2), e.g., vagus and superior laryngeal. Numerous experimenters have shown that varying the strength of the stimulus in one sensory nerve, e.g., phrenic (3), vagus (4), sciatic, crural, median, ulnar (5) or trigeminal (nasal branch) (6) may modify respiration.

Rosenthal (7) and later Meltzer (4) measured the strength of stimulus (in mm. the distance between the secondary and primary coils) necessary to bring about complete cessation of respiration in rabbits upon stimulating the central end of the cut vagus nerve. Rosenthal obtained only inspiratory inhibition of respiration but Meltzer obtained inhibition of respiration in both inspiration and expiration in the same animal upon vagus stimulation.

Bidder (8) and Blumberg (9) found, in cats, that the strength of current necessary to stop respiration in expiration upon stimulating the central end of the superior laryngeal nerve was weaker than that necessary to stop respiration in inspiration upon stimulating the vagus.

Nice (10) recently employed the Martin method (11) in studying the strength of stimulus necessary to alter respiration.

An attempt has been made in this article to answer the following three questions:

1. What are the average threshold stimuli of the vagi, superior laryngeal and cutaneous sensory nerves (such as the popliteal, peroneal, radial, ulnar and median) which bring about any change in the depth of respiration?

[1] Part of the data in this research was collected from the experiments performed upon animals in which the effect of varying the rate of stimulation upon the vasomotor centre was studied. These experiments were performed in the laboratory of Physiology in the University of Pennsylvania.

2. What average strength of current is necessary to bring about certain respiratory changes (expiratory and inspiratory decrease and complete inhibition in both phases) upon stimulating the central end of the vagus nerve?

3. What effects do different rates of stimuli have upon the respiratory centre and the threshold stimulus?

THE METHOD

These experiments were performed upon cats narcotized with urethane (2 grams per kilo by stomach) and tracheotomized. Respiratory movements were recorded by catching a small S-shaped hook in the left side of the diaphragm about midway between the central tendon and the lateral chest wall. From this hook a thread was passed over a pulley to a writing lever which wrote on a kymograph surface. The vagi were then exposed and cut and the electrodes placed centrally to the point of severance. No attempt was made to isolate the inferior laryngeal branch. After the determinations for the vagi were completed readings were made whenever possible on the following nerves in the order named; superior laryngeal, peroneal, popliteal, radial, ulnar and median nerves. Stimuli were applied by means of platinum electrodes. The nerves undergoing stimulation were protected by glass shields like those described by Sherrington (12).

The current. A glass knife blade key run by a motor made and broke the primary circuit from one to twenty times per second when the effects for changes of rate were being studied, and ten times per second for determining the effects of changes of strength of stimuli. The threshold stimulus was measured in the β units of the Martin method—Z units were also used for purposes of comparison in cases where the readings were made upon the same nerve. For these readings the electrodes were not moved after they were once in place and the tissue resistance therefore remained the same.

The strength of the current in the primary circuit was 0.1 ampere for all nerves except the superior laryngeal in which a current of 0.05 ampere was employed.

RESULTS

In 16 experiments there was a decrease in the depth of inspiration with an apparently normal depth of expiration (see fig. 1). In this figure at (*1*) the strength of the stimulus was 4.2, at (*2*) 4.6 and at

(*3*) 5 Z units. The average threshold for the whole series of experiments was 3.2 Z units and 2 β units (see table 1). To bring about the same changes upon stimulating the left vagus the average threshold stimulus was, for 10 experiments, 3.3 Z and 1.9 β units. In my experiments I did not find it necessary to leave one vagus intact as did Nice (13). In the early experiments, threshold readings were made on one vagus both before and after cutting the other. The threshold remained the same, so later both vagi were used for making readings. Nice (10) obtained slightly higher readings 2.87 and 2.52 β units and 4.15 and 4.59 Z units probably because he used slower rates of stimulation, 4 and 6 stimuli per second. Table 2 and figs. 4 and 5 are given here to show that this change in the rate of stimulation could be responsible for the increase in the threshold stimuli in Nice's experiments. In this table I used three rates of stimulation, 4 interruptions per second, at which rate the average threshold was 4.2 Z units, 10 per second with a threshold of 3.5 Z units and 20 interruptions per second with a threshold of 3 Z units. As can be seen Nice's 4.12 Z units and my readings 4.2 Z units taken at the same rate of stimulation—4 per second—are the same, considering this method accurate only within 1 or 2 per cent. This change in respiration, the production of a decrease in the depth of respiration at the expense of the inspiratory phase, is similar to the changes described by Rosenthal (14). Knoll also observed that the inspiratory movements were not as deep when the vagus was stimulated by its own demarcation current (15).

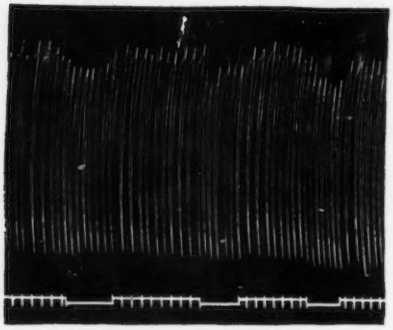

Fig. 1. Upper record tracing of respiratory movements. Lower line time in 5 second intervals and the time of stimulation. Rate of stimulation 10 interruptions per second (explained in text). In this and following curves the up stroke in the respiratory curve is inspiration, the down stroke expiration.

The superior laryngeal nerves do not require as strong a current to bring about the same response. For the right nerve the average threshold stimulus for 14 experiments was 1.5 Z and 0.8 β units and for the left the average threshold stimulus for 10 experiments was 1.3 Z and 0.7 β units (see table 1).

TABLE 1

The relative threshold stimuli (Martin Z and β units) necessary to alter the respiratory movements upon stimulating the left and right vagi and superior laryngeal nerves in the cat in urethane narcosis. Rate of stimulation ten times per second

RIGHT VAGUS				LEFT VAGUS				RIGHT SUPERIOR LARYNGEAL		LEFT SUPERIOR LARYNGEAL	
Decreased inspiration with normal expiration		Increased inspiration with decreased expiration		Decreased inspiration with normal expiration		Increased inspiration with decreased expiration		Decresed depth of inspiration with normal expiration		Decreased depth of inspiration with normal expiration	
Z	β	Z	β	Z	β	Z	β	Z	β	Z	β
1.6	0.8	2.5	1.6	2.9	2.2	5.3	4.0	1.2	0.9	1.2	0.8
2.0	1.3	4.5	3.0	1.6	0.9	4.7	3.1	1.0	0.3	1.6	1.0
2.7	1.7	5.1	3.2	3.3	1.5	6.6	3.1	1.6	1.0	1.2	0.5
3.2	1.6	7.5	3.8	3.8	2.4	5.7	3.5	1.8	0.5	1.3	0.4
5.8	3.4	7.7	4.5	2.7	1.0	6.1	2.4	1.0	0.4	1.0	0.6
6.5	4.8	13.1	9.8	4.5	2.9	11.8	8.2	1.0	0.9	1.1	0.7
1.5	1.0	2.6	1.6					1.6	0.7	1.7	0.4
0.8	0.5	2.4	1.5					1.4	0.8	1.3	0.8
1.8	1.1	4.7	3.0					1.1	0.6	1.2	0.7
2.7	1.6	5.8	3.7					1.7	1.0	1.4	0.9
2.7	1.7	4.3	2.8					1.3	0.8		
4.2	3.3			3.7	1.8			1.2	0.9		
3.3	1.9			3.3	1.9			1.6	0.8		
4.9	3.1			3.9	2.8			3.0	1.9		
3.4	1.7	5.0	2.5								
3.6	2.5										
		4.7	4.0	2.9	1.6						
Average.....3.2	2.0	5.4	3.5	3.3	1.9	6.7	4.1	1.5	0.8	1.3	0.7

TABLE 2

The threshold stimulus of the vagus as affected by changing the rate of interrruption of the stimulating current

RATE OF STIMULATION 4 PER SECOND	RATE OF STIMULATION 10 PER SECOND	RATE OF STIMULATION 20 PER SECOND
Z units	Z units	Z units
2.9	2.6	2.3
3.2	2.6	2.3
5.0	4.6	3.2
4.2	2.8	2.2
3.5	3.2	1.7
3.2	2.6	2.2
4.0	3.4	3.2
8.5	6.5	5.7
3.8	3.7	4.0
3.4	3.2	3.1
4.2	3.5	3.0

Aside from the phenomenon just described, three other effects were produced by stimulation of the central end of the cut vagus.

With an average current strength of about 6 Z units or about 4.0 β units the expiratory phase of respiration is decreased. The recording lever, therefore, does not return to the base line (see fig. 2). Here at

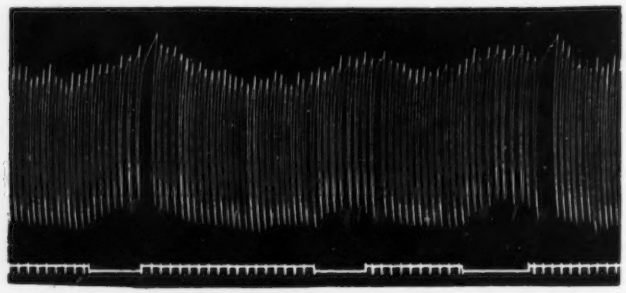

Fig. 2. Upper curve respirations. Lower line time in 5 second intervals and time of stimulations (explained in text).

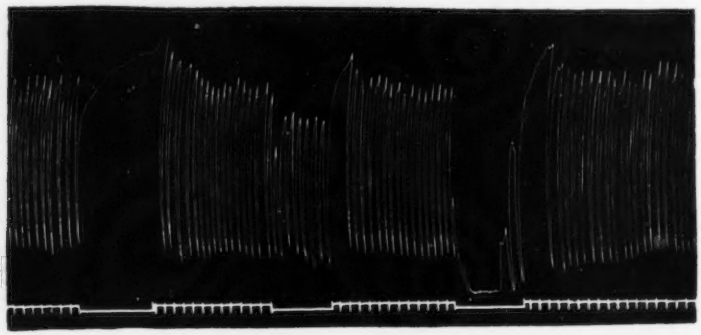

Fig. 3. Upper curve respiration. Lower line time in 5 second intervals and time of stimulation. At (*1*) the strength of the stimulus was 25 Z units, at (*2*) 59 Z units, and at (*3*) 175.5 Z units.

(*1*) the strength of stimulus was 5.7 at (*2*) 6.5 and at (*3*) 7.5 Z units. In this figure there is observed upon the cessation of stimulation an inhibition of respiration in inspiration.

As the strength of the current is further increased with a uniform rate of stimulation—10 per second—a point is reached at which respir-

ation is inhibited in inspiration. As is almost always the case considerable variation is observed in the strength of current necessary to bring about these changes in different animals. Meltzer (4) observed this same variation in his experiments upon the vagus nerve. If the nerve is not fatigued, he says, it is possible to bring about the

TABLE 3

The threshold stimulus of cutaneous sensory nerves as measured by the Martin method

NERVE STIMULATED	Z	β	AVERAGE Z	AVERAGE β
Popliteal	15.7	7.9		
	7.2	3.4		
	7.2	5.1		
	11.8	7.0		
	7.7	4.2		
	10.9	7.4		
	8.7	8.2		
	5.1	3.4	9.3	5.6
Peroneal	5.8	3.1		
	10.0	4.3		
	6.7	4.5		
	6.7	3.8		
	10.0	8.2		
	10.9	7.9	8.3	5.3
Radial	5.1	4.2		
	3.7	2.6		
	8.2	6.2		
	8.7	4.2		
	11.9	5.0	7.5	4.4
Ulnar	5.5	3.0		
	9.4	6.0		
	7.7	4.2	7.5	4.4
Median	6.6	4.1		
	11.5	9.1		
	9.4	6.5	9.4	6.6
Average	8.5	5.3	8.4	5.3

same changes in respiration repeatedly in the same animal with the same strength of current (p. 351).

In this series of experiments inhibition of respiration in inspiration was produced with 61 Z units as an average (see fig. 3). Such a re-

action was obtained in twenty-six out of twenty-nine animals experimented upon.

With a still further increase in the strength of current it was possible to bring about a cessation of respiration in expiration. The average strength of current required was 299 Z units. Thirty-one of thirty-three animals gave this response (see fig. 3). In many cases it was possible also to demonstrate this in another way. If, with a strength of stimulus sufficient to bring about inhibition of respiration in inspiration at 4 stimuli per second, the number of stimuli was increased to 20 per second the response was an inhibition of respiration in expiration (see fig. 5).

Rosenthal believed that such an expiratory inhibition was due to

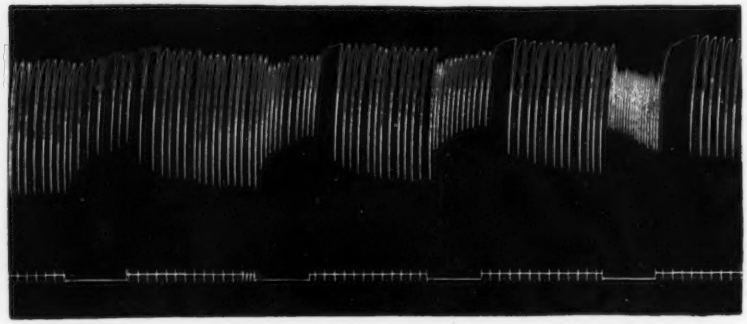

Fig. 4. Upper curve respiratory movements lower curve time in 5 second intervals and time of stimulation. Strength of stimulus 16.8 Z units. Rate of interruption, at (*1*) 4, (*2*) 7, (*3*) 10, (*4*) 20 per second.

an escape of current to the superior laryngeal nerve. Meltzer did not agree with Rosenthal and demonstrated that respiration could be stopped by a strong stimulation of the vagus. I found that if a silk ligature was tied very tightly around the vagus central to the point of stimulation, no change in respiration resulted upon stimulation although previously a complete inhibition of respiration was brought about with the same strength and rate of stimulation.

A current of only 19.5 Z units was necessary to stop respiration in expiration upon stimulating the superior laryngeal nerve. This average was taken from readings collected from 14 experiments.

The readings upon the cutaneous sensory nerves (peroneal, popliteal,

median, radial and ulnar) are considered together here. For the 25 readings made upon them the average threshold stimulus for the respiratory reflex was 8.5 Z units and 5.3 β units (see table 3).

I did not find as did Meltzer (15) that the sex of the animal experimented upon affected the response obtained upon vagus stimulation. In only five out of thirty-four animals was it impossible to obtain both inspiratory and expiratory inhibition. It was, however, very difficult and frequently impossible to bring about a complete inhibition in inspiration after inhibition in expiration had been produced.

Figures 4 and 5 are given here to show how the rate of stimulation affects the respiratory centre. In figure 4 the strength of stimulus was 16.8 Z units with a changing rate. At (*1*) the rate was 4 per second. There resulted a decrease in both the rate and depth of respiration. At (*2*) the rate was 7 per second with a resulting increase in rate and decrease in the depth of respiration. At (*3*) the rate was 10 per second and at (*4*) 20 per second each accompanied by a change in the rate and depth of respiration. Figure 5 shows a still more marked change. At (*1*) the rate of stimulation was 7 per second and the strength was 220 Z units. This caused an inhibition of respiration in inspiration. At (*2*) the rate of interruption was 20 per second with the same strength of current, 220 Z units. Here respiration ceased in expiration.

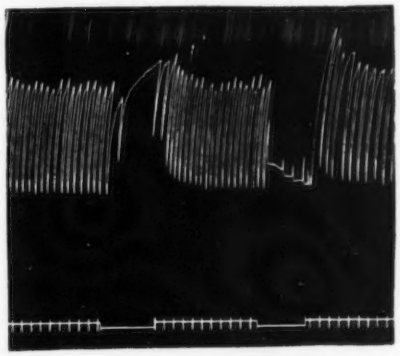

Fig. 5. Upper record respiration. Lower curve time in 5 second intervals and time of stimulation. At (*1*) rate of stimulation 7 per second. At (*2*) rate of stimulation 20 per second. Strength of stimulus 220 Z units.

Since the rate of interruption has an effect on the changes resulting from reflex excitation of the vasomotor center (16) and upon the respiratory center the question arose as to whether it might not be accountable for the difference in results obtained by Mendenhall (17) and by me upon stimulation of the cervical sympathetic (18).

Five series of threshold readings with various rates of stimuli were made using the retraction of the nictitating membrane as an index.

With a rate of 4 per second the threshold stimulus was 9 Z units, when 10 per second 7.5 and when 20 per second 5.3 Z units.

SUMMARY

1. In this series of experiments the threshold stimuli necessary to bring about a change in respiration upon stimulating the central ends of the cut vagi were 3 Z units and 2 β units for the right and 3 Z units and 1.9 β units for the left.

2. The threshold stimuli for the superior laryngeal nerves necessary to bring about a change in respiration were 1.4 Z and 0.8 β units for the right and 1.3 Z and 0.7 β units for the left. This is lower than that required by the phrenic diaphragm preparation used by Nice.

3. Sensory nerves such as the popliteal, peroneal, ulnar, radial and median nerves require a comparatively strong stimulus to effect respiration. The average stimulus was 8.3 Z and 5.2 β units.

4. A slow rate of interruption and strong stimuli have less effect upon the respiratory centre than do a high rate of interruption and a weaker stimulus.

5. The strength of stimulus required to bring about cessation of respiration in inspiration is less than that required to bring about a cessation of respiration in expiration providing the rate of stimulation remains the same. With a fixed strength of stimulus the cessation of respiration in expiration requires a faster rate of stimulation than does cessation of respiration in inspiration.

BIBLIOGRAPHY

(1) HEAD: Journ. Physiol., 1889, x, 25.
KNOLL: Sitzungsb. d. Kaiserlichen Akad. d. Wissensch. (Mathermatisch-Naturwissenschaftliche Classe), 1885, xcii, 3 Bd., 306–327.

(2) ROSENTHAL: Arch. f. Anat. und Physiol. (Physiologische Abtheilung) 1881, 39.
MELTZER: Ibid., 1892, 341.
BIDDER: Ibid., 1865, 492.
BLUMBERG: Inauge Dissert. Dorpat, 1865.
KNOLL: Sitzungsb. d. Kaiserlichen Akad. d. Wissensch., 1882, lxxxv, Bd. 3, 282.

(3) ANREP AND CYBULSKI: Pflüger's Arch. f. d. gesammt. Physiol., 1884, xxxiii, 243.

(4) MELTZER: Loc. cit.

(5) LANGENDORFF: Mittheilungen aus dem Konigsberg. Physiol. Laborat. Königsberg, 1878, 64.

(6) KRATSCHNER: Sitz. d. Wiener Akad., 1870, lxii, Bd. 2, 147.
(7) ROSENTHAL: Loc. cit. ibid., 1880, 35; ibid, 1881; ibid, 1882.
(8) BIDDER: Loc. cit.
(9) BLUMBERG: Loc. cit.
(10) NICE: This Journal, 1914, xxxiii, 204.
(11) MARTIN: Measurement of induction shocks, New York, 1912.
(12) SHERRINGTON: Journ. Physiol., 1909, xxxviii, 382.
(13) NICE: Loc. cit., 205.
(14) ROSENTHAL: Die Athembervegungen und ihre Bezeihung zum Nervous Vagus, Berlin, 1862.
(15) KNOLL: Sitzungs. d. Kaiserlichen Akad. d. Wissensch. (Mathermatisch-Naturwissenschaftliche Classe), 1882, lxxxv, Ab. 3, 282.
(16) GRUBER: This Journal, 1917, xlii, 214.
(17) MENDENHALL: This Journal, 1914, xxxvi, 60.
(18) GRUBER: This Journal, 1915, xxxvii, 259.

STUDIES IN EXPERIMENTAL GLYCOSURIA. XII

Lactic Acid Production in the Blood Following the Injection of Alkaline Solutions of Dextrose or of Alkaline Solutions Alone

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In the previous paper of this series (1) it was shown in confirmation of the work of other investigators (Murlin, Underhill) that addition of alkalies to the blood causes the percentage of blood-sugar to become lowered. There are three possible mechanisms concerned in the control of the blood-sugar level which the alkali may influence so as to bring about this change, namely: (1) the glycogenic function of the liver and muscles; (2) the oxidation of dextrose in the muscles, and (3) destruction of dextrose in the blood itself. Evidence was furnished in the above referred to communication that the alkali does not perceptibly affect the glycogenic function. In the present research we have examined the blood for the presence in it of possible break-down products of dextrose, and have chosen lactic acid as the most likely one to occur. The general plan of the investigation has consisted in the collection of samples of blood that were large enough to make an accurate determination of lactic acid possible, at intervals from the upper portion of the portal vein, or from the vena cava opposite the liver before and during the injection, lower down in the portal vein, either of dextrose solutions in the presence of a trace of acid or an excess of alkali (sodium carbonate), or of alkaline solutions without any dextrose.[1]

In the accompanying tables are shown the results of the experiments which may be divided into three groups: In the *first group* (Experiments I and II) the lactic acid was determined in blood drawn from the

[1] The details of the method used for estimating the lactic acid will be published elsewhere.

pancreatico-duodenal vein: (a) before and (b) during the injection of an alkaline solution of dextrose; (c) a few minutes after discontinuing the injection; and (d) during a subsequent injection of a similar amount of dextrose in faintly acid solution.

It will be noted that within fifteen minutes after starting the injection of alkaline dextrose solution the percentage of lactic acid in the portal blood increased in the first experiment from 0.056 to 0.096 per cent (71 per cent increase), and in the second, from 0.066 to 0.123 (86 per cent. increase). The increase is more marked in the experiment in which the dextrose was injected in moderate amount (at the rate of 0.34 gram per kilogram and hour), but the alkali was excessive (20 per cent Na_2CO_3), than in that in which the dextrose was in excess (0.73 gram per kilogram and hour) and the alkali moderate (10 per cent Na_2CO_3). This result would indicate that the factor upon which the lactic acid production depends is the alkalinity of the dextrose solution rather than the actual amount of dextrose injected. The behavior of the portal blood-sugar further corroborates this view, a much greater increase in blood-sugar being evident in the experiment (No. I) which shows the lesser increase in lactic acid.

The percentage of lactic acid was still undiminished in blood removed in ten minutes after discontinuing the injection (Experiment II). This indicates either that the lactic acid is only slowly oxidized by the animal or that the alkali continues to produce lactic acid from the dextrose of the blood, or from that injected along with the alkali, for some time after discontinuing the injection. It is also possible that breakdown products of the action of alkali on dextrose continue to circulate in the blood for some time after the blood has regained its normal H-ion concentration, and that these only become converted into lactic acid as some of this becomes oxidized. In other words, the lactic acid after accumulating to a certain extent may limit any further production in obedience to the laws of mass action.

In both experiments, following a period during which no injection was made, dextrose was again injected, but in faintly acid solution (2 to 3 drops concentrated HCl per 100 cc. of solution). In Experiment I, although distinctly more dextrose was injected than during the previous alkali period, the lactic acid percentage was found to be about 10 per cent lower. In the light of results which we will discuss immediately it is probable that the increase which does exist in this part of the experiment would have been much less evident had a longer period of time been allowed to elapse following the injection of alkali.

In Experiment II, the lactic acid percentage during the injection of acid remained at its previous high level, a result which is in harmony with the above stated view, namely that there still remained in the blood of this animal an accumulation of lactic acid or of some precursor produced by the action of the alkali.

In the *second group* (Experiments III and IV), the dextrose was first of all injected in faintly acid or neutral solution and later in the presence of alkali.

In Experiment III a 5 per cent solution of the purest dextrose obtainable (Merck's) was injected in the usual way, first of all in faintly acid solution and later in alkaline. The rate of injection was a little quicker during the latter as compared with the former period. On account of omission to record the weight of the animal, the exact dosage per kilogram body weight cannot be given, but we know that it was well within the utilization value of 0.8 gram per kilogram an hour. Lactic acid was determined in blood removed as nearly as possible simultaneously from the portal vein and vena cava, the object of this being to see whether any of the lactic acid produced in the portal blood would be retained by the liver. The blood-sugar was determined by the Bertrand method in aliquot portions of the Schenck filtrate, the remainder of which was used for estimation of the lactic acid.

It will be noted that practically no increase in lactic acid was produced in the blood of either vessel, as a result of the injection of dextrose in acid solution,[2] whereas, on the other hand, an increase from 0.039 to 0.076 (95 per cent) occurred in the blood of the portal vein, and from 0.050 to 0.092 (85 per cent) in that of the vena cava, following the injection of the alkaline solution. The increase in dextrose-content was only moderate and approximately the same during both periods.

Two conclusions may be drawn from the results of this experiment: first, that the injection of a faintly acid solution of chemically pure dextrose does not cause any perceptible increase to occur in the lactic acid content of the blood, and secondly, that when such an increase does occur as a result of the injection of practically the same amount of pure dextrose dissolved in alkali, the lactic acid thus formed is not retained by the liver. Why there should have been somewhat more

[2] Some loss is known to have been incurred in this estimation on account of clotting of blood during its collection.

lactic acid in the blood of the vena cava than in that of the portal vein, we cannot explain.

In Experiment IV, the procedure was in general the same as that of Experiment III, except that blood for analysis was removed from the portal veing alone (an unmanageable clot having formed in the vena cava cannula). The dextrose was not so pure a specimen as that used in the previous experiment (Mulford's), and it was injected at first in neutral reaction and later in the presence of 15 per cent Na_2CO_3.

It will be noted that the lactic acid content was initially high in this animal, and that if anything it was very slightly increased (from 0.065 to 0.067 per cent) by the injection of a neutral solution of dextrose at a rate of 0.36 gram per kilogram and hour. The injection of the alkaline solution was then started at a rate which was at first only about two-thirds that of the neutral solution (0.26 gram per kilogram and hour), and yet, within ten minutes after starting the injection, by which time 3 grams Na_2CO_3 had been injected, the lactic acid percentage had increased from 0.064 to 0.074 (15 per cent increase). The injection rate was then greatly increased (to 2.5 gram dextrose per kilogram and hour, with a total injection of 6 grams Na_2CO_3), with the result that within three minutes the lactic acid percentage had mounted from 0.074 to 0.095 (29 per cent increase over previous estimation or 46 per cent over normal).

In Experiment V, dextrose was injected in large dosage (over 1 gram per kilogram and hour) into the mesenteric vein, blood for analysis being collected from the vena cava. During the first period of about 15 minutes a dextrose solution which was faintly acid in reaction caused no increase in the lactic acid percentage of the blood. In ten minutes after discontinuing the injection, however, the percentage had risen from 0.05 to 0.065. A similar amount of dextrose in sodium carbonate solution was then injected, with the result that in about ten minutes the lactic acid percentage of the blood had almost doubled (from 0.065 to 0.125). The results of this experiment corroborate those recorded above in two particulars: first, the dextrose injected in acid solution causes no increase in the lactic acid content of the blood, whereas a similar dextrose injection in alkaline solution causes a marked increase; and secondly, that the lactic acid thus produced is not retained by the liver.

Most of the above observations were made during the course of experiments which were primarily designed to study the behavior of the blood-sugar level during the injection of dextrose solutions in the pres-

ence of acid or alkali. On this account the experiments on the effect on the lactic acid content of the blood of injections of alkali alone which form the experiments of *group three* were not performed until quite recently, and from the results (Nos. VI and VII in the table) it is plain that the lactic acid is actually produced in the blood itself and is not, as we had thought probable, dependent upon the presence in the injected alkaline dextrose solutions of decomposition products. Thus, by the injection of Na_2CO_3 in 15 per cent solution at the rate of 0.6 gram per minute into a dog weighing 8.7 kilogram (Experiment VI), the lactic acid per 100 cc. blood increased, after twelve minutes' injection, from an average of 0.030 gram to 0.063 gram, and after fifteen minutes, it had risen further to 0.076 per cent. In the other experiment of this nature (No. VII) the alkali was injected a little quicker (viz., 0.66 gram per minute in a somewhat smaller dog) with the result that in six minutes the lactic acid content of the blood rose from 0.033 to 0.093, at about which level it also stood in eleven minutes.

These are of course very large amounts of alkali, but they are comparable with those of the dextrose solutions used in the previous experiments. There can be little doubt that a distinct increase in lactic acid of the blood would also be produced by the injection of much less of the carbonate, but we have not as yet determined just exactly how much must be injected in order to cause a measurable increase.

It is possible that more lactic acid would be produced by injections of dextrose plus alkali than by alkali alone. If dextrose be the source of the acid, such would be the expected result, and although the alkaline solutions of dextrose do not, on standing for a short time at room temperature, form any detectable amount of lactic acid, yet there is evidence from the "browning" of the solutions that some considerable decomposition of the dextrose molecule has set in. It may be that certain of the decomposition products are very promptly acted on by some enzyme present in blood (of the glyoxylase class) to form lactic acid.

That the addition of weak alkali to sterile blood *in vitro* increases the rate of disappearance of dextrose (2), and its addition to suspensions of leucocytes causes lactic acid to be produced from dextrose (3) are facts which have an interesting bearing on our results.

There are many interesting phases of the problem which await further investigation. We have placed the above results on record at the present time because of the bearing which they have in connection with the influence of alkali injections on blood-sugar.

BIBLIOGRAPHY

(1) MACLEOD AND FULK: This Journal, 1917, xlii, 193.
(2) RONA AND DÖBLIN: Biochem. Zeitschr., 1911, xxxii, 489; and Rona and Arnheim: ibid, 1913, xlviii, 35.
(3) LEVENE AND MEYER: Journ. Biol. Chem., 1912, xi, 347 and xii, 265.

TABLE

NO. AND GENERAL NATURE OF EXPERIMENT	WEIGHT OF DOG	SUBSTANCE BEING INJECTED INTO MESENTERIC VEIN DURING PERIOD	DURATION OF PERIOD	VESSEL FROM WHICH BLOOD COLLECTED FOR ESTIMATION	DEXTROSE IN BLOOD (AVERAGE OF SEVERAL SAMPLES COLLECTED DURING PERIOD)	LACTIC ACID (IN SAMPLES COLLECTED DURING PERIOD)	PERCENTILE CHANGE FROM NORMAL
	kg.		*min.*				
I. Alkaline, then faintly acid dextrose solution injected	13.4	*a.* No injection		Pancreatico duodenal	0.115	0.056	
		b. 0.73 gram dextrose per kilogram, hour, *plus* 3.2 gram Na_2CO_3 in 10 % solution	15	Pancreatico duodenal	0.195	0.096	71 % increase
		c. No injection	15	Pancreatico duodenal	0.137		
		d. 1.07 gram dextrose per kilogram hour, *plus* trace of HCl	10		0.195	0.87	55 % increase
II. Alkaline, then faintly acid dextrose solution injected	20	*a.* No injection		Pancreatico duodenal	0.078	0.066	
		b. 0.34 gram dextrose, (in 4 % solution) per kilogram, hour, *plus* 8 gram Na_2CO_3 in 20 % solution	14	Pancreatico duodenal	0.110	0.123	86 % increase
		c. No injection	10	Pancreatico duodenal	0.098	0.121	83 % increase
		d. 0.32 gram, dextrose (in 4 % solution) per kilogram, hour, *plus* trace HCl	10	Pancreatico duodenal	0.149	0.125	89 % increase

III. Faintly acid, then alkaline dextrose solution injected		*a.* No injection		Pancreatico duodenal	0.119	0.039	
				Vena cava	0.093	0.050	
		b. 0.085 gram dextrose per minute in faintly acid solution*	10	Pancreatico duodenal	0.131	0.043	11 % increase
				Vena cava	0.152	0.041	18 % increase
		c. 0.10 gram dextrose per minute *plus* 2.8 gram Na_2CO_3 in 10 % solution†	13	Pancreatico duodenal	0.146	0.076	95 % increase
				Vena cava		0.092	85 % increase
IV. Neutral, then alkaline solution injected	26.9	*a.* No injection		Pancreatico duodenal	0.087	0.065	
				Vena cava		0.062	
		b. 0.36 gram per kilogram hour in neutral solution	22	Pancreatico duodenal	0.167	0.067	3 % increase
			25	Pancreatico duodenal		0.064	
		c. 0.26 gram dextrose per kilogram, hour, *plus* 3 gram Na_2CO_3 in 15 % solution	9	Pancreatico duodenal		0.074	15 % increase
		d. 2.5 gram dextrose per kilogram, hour, *plus* 6 grams Na_2CO_3 in 15 % solution	3	Pancreatico duodenal	0.178	0.095	46 % increase
V. Faintly acid, then alkaline dextrose solution injected	8.8	*a.* No injection		Vena cava	0.063	0.045	
		b. 1.02 gram dextrose per kilogram, hour, *plus* trace of HCl	15	Vena cava	0.013	0.050	10 % increase
		c. No injection	10	Vena cava	0.105	0.065	44 % increase
		d. 1.05 gram dextrose per kilogram, hour, *plus* about 5 grams Na_2CO_3 in 10 % solution	13	Vena cava	0.125	0.125	177 % increase

* Air emboli in injection fluid.

† Blood pressure falling rapidly.

NO. AND GENERAL NATURE OF EXPERIMENT	WEIGHT OF DOG	SUBSTANCE BEING INJECTED INTO MESENTERIC VEIN DURING PERIOD	DURATION OF PERIOD	VESSEL FROM WHICH BLOOD COLLECTED FOR ESTIMATION	DEXTROSE IN BLOOD (AVERAGE OF SEVERAL SAMPLES COLLECTED DURING PERIOD)	LACTIC ACID (IN SAMPLES COLLECTED DURING PERIOD)	PERCENTILE CHANGE FROM NORMAL
	kg.		*min.*				
VI. Alkali alone injected	8.7	*a*. No injection		Pancreatico duodenal	0.182	0.020	
					0.174	0.033	
		b. Injection of 0.6 gram per minute Na_2CO_3 in 15 % solution	12	Pancreatico duodenal	0.186	0.063	110 % increase
		c. Injection of 0.6 gram per minute Na_2CO_3 in 15 % solution	15			0.076	153 % increase
VII. Alkali alone injected	7.2	*a*. No injection		Pancreatico duodenal	0.091		
			5	Pancreatico duodenal	0.084	0.033	
		b. Injection of 0.66 gram per minute Na_2CO_3 in 15 % solution	6	Pancreatico duodenal	0.093	0.093	181 % increase
		c. Continuation of same injection	11	Pancreatico duodenal	0.054	0.088	166 % increase

FURTHER STUDIES OF NERVE CONDUCTION IN CASSIOPEA

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Studies of recent years have shown the importance of hydrogen ion concentration in determining the rate of nerve conduction in the medusa *Cassiopea*. Ordinary distilled water often remains acid even though air, freed from CO_2 by means of soda-lime, has been bubbled through it for seventy-two hours. Accordingly, Prof. George A. Hulett kindly arranged to have 144 liters of distilled water prepared in accordance with his well known method (see Hulett (1)), in his laboratory at Princeton University. This water was sealed in 144 pyrex glass flasks and thus transported to Tortugas. The hydrogen ion concentration of each flask was testedseparately, the range being 0.8 to 1×10^{-6}, and the average being 0.9×10^{-6}, or 6.04 PH.

Fifty litres of this water were placed in a green glass carboy which had previously held Merck's distilled water; and air freed from CO_2 was bubbled through it at an active rate for seventy-eight hours, after which the water in the carboy had a hydrogen ion concentration of 10^{-8} which it maintained for eight days while 139 experiments were made with it. The alkalinity then declined to a hydrogen ion concentration of 3.16×10^{-8} while 26 other experiments were made; the average hydrogen ion concentration for the series being 1.17×10^{-8}, or 7.93 PH. Its alkalinity was probably due to soda derived from the glass carboy, the balance being maintained by a tendency of the water itself to become acid through leakage of CO_2 from the air. Prof. J. F. McClendon found the PH of the Tortugas sea water to range from 8.1 to 8.22 (0.794 to 0.603×10^{-8} CH); and dilution with alkaline distilled water of hydrogen ion concentration 1.17×10^{-8} maintained the normal alkalinity of the sea water even when diluted with its own volume of such distilled water.

The electrical conductivity of the sea water was determined by Kohlrausch's method, using a telephone to detect the minimum sound from an alternating current produced by a small induction coil.

It appears that when alkaline distilled water of about 8 PH is used to dilute the sea water the decline in rate of nerve conduction is practically the same as the decline in the electrical conductivity of the sea water when similarly diluted. We should remember, however, that the concentration of the sodium, calcium and potassium cations declines in practically the same ratio and we believe we can show that the rate of nerve conduction is proportional to the concentration of these cations rather than to the electrical conductivity of the diluted sea water as a whole.

TABLE 1

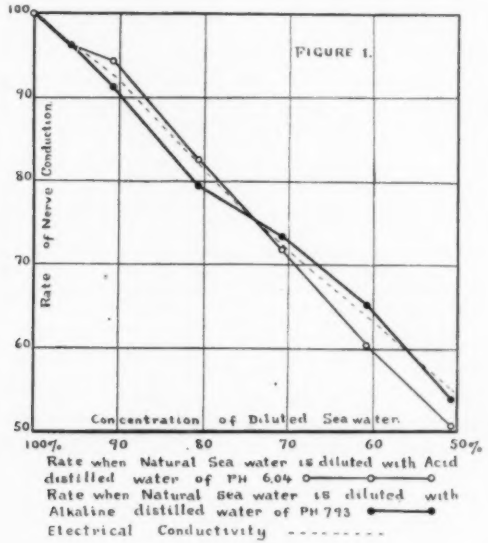
Illustrated by figure 1

Rates of nerve conduction when Tortugas sea water is diluted with aerated, alkaline, distilled water having a hydrogen ion concentration of 1.17×10^{-8}

COMPOSITION OF THE SOLUTION	RATE OF NERVE CONDUCTION	ELECTRICAL CONDUCTIVITY OF TORTUGAS SEA WATER DILUTED WITH DISTILLED WATER OF HYDROGEN ION CONCENTRATION 1.58×10^{-8} AT 30°C.
Natural sea water	100.00	100.00
95 volumes sea water + 5 volumes distilled water	96.23	
90 volumes sea water + 10 volumes distilled water	91.44	92.16
80 volumes sea water + 20 volumes distilled water	79.51	81.38
70 volumes sea water + 30 volumes distilled water	73.91	71.53
60 volumes sea water + 40 volumes distilled water	65.72	64.26
50 volumes sea water + 50 volumes distilled water	54.16	54.08

However, Lillie (2) is right in stating that the rate of nerve conduction in *Cassiopea* in diluted sea water does not decline in accord with Freundlich's law of adsorption as I had erroneously supposed. The distilled water used in my previous experiments was slightly acid and this appears to be stimulating in slight dilution, becoming depressant, however, as the dilution is continued, thus giving the semblance of an adsorption curve. Nor did I realize the effects of slight changes in temperature in the various solutions, for the effect of 0.1°C. is readily appreciable.

Lillie advocates an extension of the theory of Faraday and de la Rive, that the transmission of the excitation state from the immediate site of activity to the adjoining resting area is dependent on an electrical local action of the same essential nature as that which is responsible for the etching or corrosion of non-homogeneous metallic surfaces, such as iron in contact with an electrolyte solution; and if this be true, the rate of nerve conduction should be a function of the electrical conductivity of the medium, and also of the surrounding fluid.



To further test this hypothesis, I determined the electrical conductivity of Tortugas sea water when heated or cooled, and compared it with the rate of nerve conduction at corresponding temperatures.

This shows that the rate of nerve conduction has a temperature coefficient about two and a half times as high as that of the electrical conductivity of sea water; as will appear in table 2.

It will be recalled that Harvey (3) was the first to show that the rate of nerve conduction in *Cassiopea* augments in nearly a straight line as the sea water is heated until about 36° to 38°C. where it suddenly falls off, thus giving a curve resembling that of an enzyme reaction. This has been confirmed by the later work of Mayer (4), and Cary;

and I find that when the rate has begun to fall off, the original rate is not recovered upon cooling. This may possibly mean that the hypothetical enzyme has been partially destroyed; for if this decline were due

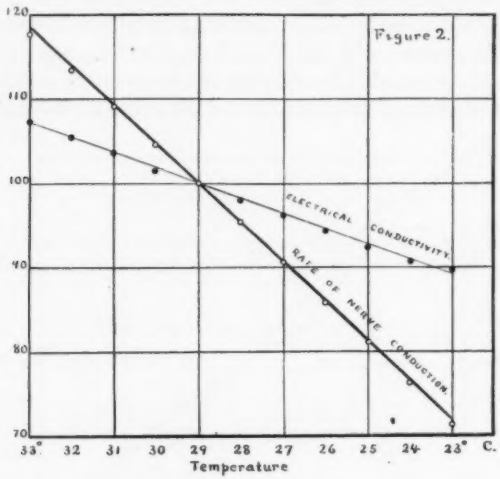


TABLE 2

Illustrated by figure 2

TEMPERATURE C° OF THE SEA WATER	RATE OF NERVE CONDUCTION IN CASSIOPEA. MEAN OF HARVEY, MAYER AND CARY'S OBSERVATIONS	RELATIVE ELECTRICAL CONDUCTIVITY OF TORTUGAS SEA WATER DETERMINED BY KOHLRAUSCH'S METHOD
23	71.30	88.9
24	76.30	90.7
25	81.17	92.6
26	85.80	94.4
27	90.74	96.2
28	95.47	98.0
29	100.00	100.0
30	104.47	101.6
31	109.20	103.5
32	113.40	105.3
33	117.80	107.1

merely to Winterstein's heat asphyxiation (5) the rate should recover to a greater degree when the medusa is replaced in sea water of normal temperature. Moreover, the high temperature coefficient of the rate

of nerve conduction suggests that we may be dealing with a chemical reaction in which a compound is formed composed of sodium, calcium and some proteid element, (Osterhout (6)), the degree of ionization of which is considerably affected by temperature in the manner suggested by Hardy (7), Quincke (8) and Bayliss (9).

Possibly, also, the negative electrical potential associated with the wave of nerve conduction may increase the surface tension of the alkaline colloidal particles thus reducing their size, rendering them more soluble and thereby increasing the concentration of the reacting ions. (See Mayer, Schaeffer and Terroine (10); and also Hulett (11)).

Contrary to Lillie's hypothesis, we have direct evidence that the rate of nerve conduction may be independent of the electrical conductivity of the surrounding sea water, for I have found (12) that if sea water be diluted with 0.415 molecular $MgCl_2$ the rate of nerve conduction is only slightly more depressed than if the sea water is diluted with distilled water, yet the $MgCl_2$ maintains a nearly normal electrical conductivity, while with distilled water it declines in nearly the same ratio as the dilution. Nor do the experiments I have made with solutions containing some, but not all the cations of sea water, support Lillie's view. For example, if the rate of nerve conduction in 0.647 molecular NaCl is 55, it becomes 100 in 85.3 cc. of 0.6 molecular NaCl + 14.69 cc. of 0.39 molecular $MgCl_2$. Here the electrical conductivity of the solution is somewhat reduced while the rate of nerve conduction is much augmented. This is, of course, a striking instance of Loeb's law of the antagonism between a univalent and a bivalent cation; even though the bivalent cation in this case is magnesium, well known to be a depressant especially for muscular activity in *Cassiopea*.

In slight dilution such as 90 per cent sea water + 10 per cent of distilled water, acid distilled water of hydrogen ion concentration 10^{-6} at 30°C. is slightly stimulating when compared with the effect of alkaline distilled water of 10^{-8} hydrogen ion concentration. In dilution of about 30 per cent distilled water + 70 per cent sea water, and greater, the effect of the acid sea water is reversed for it becomes more and more depressant. This fact will appear upon inspection of table 3.

McClendon (13) found that even when sea water of 8.1 PH is mixed with an equal volume of distilled water of 6 PH the resulting mixture has a PH of 8.09. Thus it is improbable that the stimulating effect of slight amounts of this acid distilled water is due to a change in the concentration of hydrogen ions, especially as the effects of any decided concentration of acid are well known to be depressant.

It seems more probable that the phenomenon is comparable with that observed by Osterhout (14) in plants wherein a weak concentration of HCl is at first stimulating but later after the plasma membrane has been penetrated by the acid it becomes depressant. A slight accession of H ions not sufficient to appreciably change the alkalinity of the sea water appears to be stimulating; and it is possible that it may be unable to penetrate the cell and produces its effect by simple contact with the surface membrane. In stronger concentrations it certainly penetrates the cell and then becomes a depressant for physiological activity.

There are remarkable parallels between the physiological reaction of animals and plants indicating the fundamental kinship of the phenomena. Thus Osterhout (6) has ingeniously calculated the amount of

TABLE 3

Illustrated by figure 1

COMPOSITION OF THE SOLUTION	RATE OF NERVE CONDUCTION WHEN SEA WATER IS DILUTED WITH ACID DISTILLED WATER OF 0.9×10^{-6} H ION CONCENTRATION AT 30°C.	RATE OF NERVE CONDUCTION WHEN SEA WATER IS DILUTED WITH ALKALINE DISTILLED WATER OF 1.17×10^{-9} H ION CONCENTRATION AT 30°C.
Natural sea water of 8.1 to 8.2 PH	100	100
95 cc. sea water + 5 cc. distilled water	96.43	96.23
90 cc. sea water + 10 cc. distilled water	94.4	91.44
80 cc. sea water + 20 cc. distilled water	82.7	79.51
70 cc. sea water + 30 cc. distilled water	71.95	73.91
60 cc. sea water + 40 cc. distilled water	60.41	65.72
50 cc. sea water + 50 cc. distilled water	50.83	54.16

the so-called sodium-calcium-ion proteid formed in *Laminaria* when placed in solutions containing NaCl and $CaCl_2$ mixed in various proportions. He shows that a toxic monivalent cation, such as sodium, causes a fall of resistance, but if a bivalent cation such as calcium be also added the fall of resistance is inhibited. Thus the amount of antagonism may be quantitatively determined.

Lillie's hypothesis of electrolytic local action appears to me to be too simple to account for the facts of nerve conduction. As Professor McClendon says there is no such optimum temperature for electrical conductivity, as we observe at about 36°C. for nerve conduction. Nerve reactions must indeed by very much more complex than the "local action" theory would imply, for Cary (15) finds that even if a single

one of the 16 or more marginal sense-organs of *Cassiopea* be intact the initial stages in regeneration are decidedly accelerated in comparison with the rate observed when *all* of the sense-organs are removed.

SUMMARY

Nerve conduction is due to a chemical reaction involving the cations of sodium, calcium and potassium; magnesium being non essential to the reaction.

The sodium and calcium cations probably combine together with some proteid element to form a sodium-calcium-ion proteid. The temperature coefficient for the rate of nerve conduction is two and a half times as great as that for the electrical conductivity of the sea water surrounding the nerve, and this may be due to a high temperature coefficient of ionization of the sodium-calcium-ion proteid, which is concerned in the chemical reaction resulting in nerve conduction.

The rate of nerve conduction may be independent of the electrical conductivity of the solution surrounding the nerve, for the rate declines in practically the same manner whether we dilute the sea water with 0.4 m. $MgCl_2$ or with distilled water. In other words whether we maintain the electrical conductivity or reduce it in a ratio nearly commensurate with the dilution.

BIBLIOGRAPHY

(1) HULETT: Zeitschr. phys. Chemie, 1896, xxi, 287.
(2) LILLIE: This Journal, 1916, xli, 133.
(3) HARVEY: Papers from Tortugas Laboratory of Carnegie Institution, 1911, iii, 27.
(4) MAYER: Papers from Tortugas Laboratory of Carnegie Institution, 1914, vi, 8.
(5) WINTERSTEIN: Zeitschr. für allg. Physiol., 1905, v, 323.
(6) OSTERHOUT: Proc. Amer. Phil. Soc., 1916, lv, 533.
(7) HARDY: Journ. Phys. Chem., 1900, iv, 236.
(8) QUINCKE: Ann. Phys., 1902, ix, 1912.
(9) BAYLISS: Principles of general physiology, 1915, 77.
(10) MAYER, SCHAEFFER ET TERROINE: Compt. Rend. Paris, 1907, cxlv, 918.
(11) HULETT: Zeitschr. phys. Chem., 1901, xxxvii, 406.
(12) MAYER: Proc. Nat. Acad. Sci., 1915, i, 270. Also this Journal, 1916, xxxix, 381.
(13) MCCLENDON: Proc. Nat. Acad. Sci, 1916, December, ii. 689.
(14) OSTERHOUT: Journ. Biol. Chem., 1914, xix, 518.
(15) CARY: Proc. Nat. Acad. Sci., 1915, i, 611.

THE NATURE AND TIME RELATIONS OF THE FUNDAMENTAL HEART SOUNDS

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I. ARGUMENT

During each cardiac cycle, at least two series of vibrations, of such a frequency that they can be distinguished by the human ear, are initiated within the heart wall, the valves and blood current. They may be designated as the *fundamental heart sounds.* Through the air and solid media of the intervening lung these vibrations are communicated to the thoracic wall. The resultant thoracic vibrations, and not the fundamental cardiac vibrations, are heard in auscultation and recorded by instrumental means. They may be differentiated by the term *thoracic heart sounds.*

In studying the causes, characteristics and chronotropic relations of the sounds by recording mechanisms it is important to have accurate records of the fundamental vibrations. It is questionable, however, whether the vibrations recorded from the thoracic wall are duplicates of the fundamental vibrations originating within the heart. There are theoretical as well as experimental reasons for believing that vibrations may be deleted or added to the fundamental vibrations before they reach the receiver placed upon the chest. As the amplitude of sound vibrations decreases directly with the distance that they are propagated, it is conceivable that vibrations of small amplitude may fail to reach the thoracic wall or become too small to be recorded. The extent to which depression occurs in the apex region depends on the volume of intervening lung, which varies not only in different subjects but, in the same individual, with the phases of respiration. In this way Fahr (1) and Battaerd (2) have explained the frequent variations in contour and grouping of the first sound vibrations taken from the apex region. Owing to the greater distance between the base of

the ventricles and chest wall, even larger fundamental vibrations originating in the aorta and pulmonary artery may fail to reach the thoracic wall. Consequently, sound vibrations are not only more difficult to record from the second interspaces but are subject to even greater variation in grouping and contour (2).

Vibrations may be added to the fundamental vibrations in several different ways. In the first place, adventitious respiratory sounds may be incorporated with the fundamental vibrations. This may occur even during expiratory apnea for each change in the volume and position of the ventricles exerts a cardio-pneumatic effect on the lungs capable of creating sound vibrations within lung tissue. In the second place, the thoracic wall may be thrown into vibration by mechanical impacts, as is well known to be the case in thoracic percussion. Conditions for inaugurating such an inherent vibration of the chest wall are especially favorable at the onset of ventricular systole (i.e., during the first sound) owing to the fact that at this time the ventricular apex gives an impact to the thorax. How important this factor is can not be established until the vibration frequency and decrement of the inherent thoracic vibrations are known. That it varies greatly with the type of thorax and vigor of the apex impulse must be logically conceded.

Finally, the relative amplitude of the transmitted fundamental vibrations may be altered. The heart sounds are composed of vibrations varying greatly in their individual periods. The air-containing thorax, which acts as a resonator, may therefore intensify the amplitude of those vibrations with which it is capable of vibrating in unison and reproduce them out of proportion to other vibrations which differ essentially from the inherent thoracic period. In these ways the configuration of the sound groups may be altered or certain waves entirely repressed.

II. METHOD OF RECORDING FUNDAMENTAL HEART SOUNDS

In order to study the fundamental heart sounds as they occur in the heart, we devised a sound transmitter capable of picking up the sound vibrations from any point of the exposed heart and transmitting them to a sensitive recording membrane.

The idea of our sound transmitter was suggested through the previous use of a miniature myocardiograph described elsewhere by one of us (3). Records taken with this apparatus from the contracting

auricle often gave an indication of sound vibrations superimposed on the myogram curve. When such a myocardiograph was connected to a very sensitive recording capsule and a side tube in the system was left open, sound vibrations were recorded from any portion of the heart. In order to eliminate to a considerable extent the myogram element, the use of the fixed arm was discontinued, the apparatus being attached to the heart by the movable arm alone. In order to increase the inherent frequency, the segment capsule to which the movable limb was attached was covered by tensely drawn, heavy rubber dam. Thus transformed, the apparatus shows the essential parts diagrammatically illustrated in figure 1 A. The arm, *a*, is fastened by a stitch to any portion of the heart; e.g., ventricle, auricle or aorta. Several of these instruments may be applied simultaneously to the heart in this way, the only limitation in number being that contact of the different transmitters with each other or with other apparatus must be avoided.

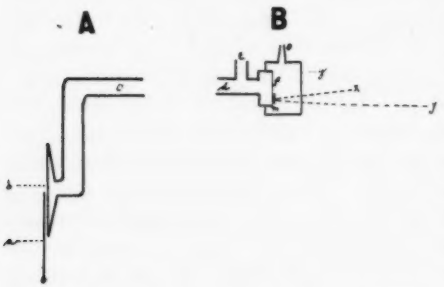

Fig. 1. Diagram showing essential parts of sound recording apparatus. A, sound transmitter; *a*, arm stitched to heart and communicating with diaphragm, *b;* B, protected recording capsule; *d*, receiving tube with side opening, *e*, to eliminate mechanical variations in the system; *f*, rubber film, supporting mirror, *m*. Capsule is inclosed by housing with front plane window, *g*, and a vent, *o*, for equalizing pressures on membrane.

The sound transmitter is connected by soft rubber tubing about 30 cm. long[1] with a Frank capsule which is 10 mm. in diameter and covered with a delicate rubber film. This film is made by passing the opening of the capsule through a strand of rubber cement and subsequently stretching the partially dried film in order to render it tense. Before the film has completely dried, a tiny mirror (1 to 2 mm. square) is placed upon it and allowed to adhere. Such a membrane exposed to the air is responsive not only to sound vibrations communicated by the tube, *d*, but is so sensitive to accidental sound waves which impinge upon the side exposed to the room that, at certain times, it

[1] This short tube introduced a delay of only 0.00099 seconds in sound transmission.

is almost impossible to obtain clear heart sound records. To obviate this objection, our earlier experiments were carried on only at night when accidental noises were reduced to a minimum. Recently, it has been found possible, however, to modify the apparatus in such a way that it does not respond to accidental room vibrations of considerable intensity. This modification consists in inclosing the membrane by a housing, *g*, having a front window of perfectly plain glass. In this housing is a conical vent, *o*, tapering exteriorly to an aperture only 1 mm. in diameter. This is sufficient to equalize the slight pressure variations within the housing but prevents the entrance of extraneous sound vibrations.

III. THE EFFICIENCY AND LIMITATIONS OF THE SOUND RECORDING MECHANISM

It is of primary importance to determine whether the sound recording system utilized is capable of responding faithfully to all the sound vibrations that may originate within the heart and, above all, to satisfy ourselves that the errors attributed to the interposition of the thoracic wall are not reduplicated by the recording system. In relation to this question the vibration frequency is of paramount interest. The complete system had a vibration frequency ranging from 150 to 250 per second, depending on the tensión of the rubber stretched over the transmitter, as well as on the tension given the rubber film of the recording capsule. Care was taken that the frequency did not fall below the former figure but it was not technically possible at the time these experiments were made to increase the frequency above 250 per second. While such a system is capable of giving *an indication* of vibrations occurring at any frequency not exceeding its inherent rate, it is not capable of vibrating with an amplitude devoid of resonance effects when the frequency exceeds about 100 per second. This was established by its ability to record tuning-fork vibrations of different amplitude and period.

A consideration of the average frequency of the sound waves recorded from man up to the time that this research was inaugurated, led to the conclusion that the sounds of the heart are composed of vibrations having a frequency less than 100 per second. Thus, Gerhartz (4) calculated that the average frequency of the first sound in Einthoven's curves was 39.4 and of the second sound, 47.5 per second, while Einthoven (5) himself gave 50 per second as the frequency of

the third sound. Frank (6) and Lewis (7) found that the average frequency of the two sounds approximated 40 per second although the latter observer sometimes found frequencies as high as 70 per second for the first, and 86 per second for the second sound. Consequently, at the time these experiments were undertaken, we were under the impression that these capsules were capable of recording without error all the individual vibrations occurring within the heart.

Recently, Battaerd (2) has found that some of the sound vibrations taken from man by a galvanometer string capable of vibrating 3000 times per second have a period as short as 0.001 second. It is quite evident that our capsules are entirely incapable of responding to vibrations even approximating such a figure. It may be emphasized, therefore, that our description of the vibrations occurring within the heart is limited to the predominant vibrations of longer period. Whether additional vibrations of shorter period actually exist within the heart or whether the short vibrations found in the thoracic sounds by Battaerd are due to overtones or harmonics originating in the thoracic cavity, must be subsequently determined by other methods.

After considerable experimentation we came to the conclusion that it is unwise to adopt special means of increasing the damping of the capsules. Consequently, they are not quite aperiodic. This introduces the possibility that any sudden, mechanical impact derived from the heart may set the system into vibration at its own inherent rate. Experimental tests, however, indicate that this does not occur when an adequate side opening is maintained in the system. A sound transmitter was attached by a thread to a 100 per second tuning-fork which was so mounted that its movements were simultaneously recorded with the corresponding vibrations of the optical capsule. If the vibrating tuning-fork was then rapidly moved through a distance much greater than is possible when attached to the heart no inherent vibrations were added to the sound record and the periods of vibrations remained unaltered, the only change being a modification of the relative amplitude of the recorded sounds (fig. 2). This may be explained by the fact that the decremental quotient[2] of the inherent vibrations was favorable. Thus, in a typical experiment the quotients 1.54, 1.33, 1.33, 1.37, 1.23, 1.41 and 1.5 (average 1.38) were found in comparisons of successive vibration phases. The average

[2] By the *decremental quotient* is understood the quotient obtained by dividing the amplitude of any vibration phase by the amplitude of the next vibration phase.

quotients found in the capsules used at different times ranged from 1.2 to 1.48.

In addition to these instrumental checks upon the efficiency of the apparatus, many precautions were taken during the experiments in order to prevent the communication of accidental vibrations to the transmitter or connecting tubing. The sound transmitter attached to the heart was suspended by its horizontal arm through a ring of very thin rubber, which enabled the entire apparatus to follow the movements of the heart readily and yet give the swing of the entire instrument a very long period. The connecting tube was similarly suspended so that its sway also had a low frequency. To annul vibra-

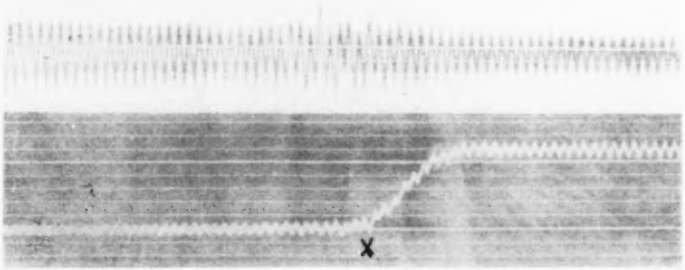

Fig. 2. Curve showing the effect of a sudden movement of a vibrating tuning fork at x (lower curve) on the vibrations recorded by an optical capsule (upper curve).

tions of pulmonary origin the records were taken during the apnea following a temporary lull in artificial respiration during which phase the lungs were completely collapsed. To avoid friction rubs of the moving heart, this organ was allowed to remain intact within the pericardium, a small window only being cut over the point to which the arm of the sound transmitter was attached. In the earlier experiments the entire animal and operating boards were also suspended by appropriate springs in order to avoid the transmission of vibrations by way of the building. This precaution was later found to be unnecessary since these vibrations apparently are damped by the resiliency of the animal's own body.

Finally, the efficiency of the system was checked by auscultation just before records were taken. If a stethoscope is connected with the sound transmitter each sound has a quality not unlike that heard

over the thoracic wall. Records were taken only when the sounds were clear and free from friction rubs or other accessory noises. In one instance the records obtained by the optical capsules were also compared with those obtained by substituting a microphone circuit connected with a highly damped galvanometer string capable of vibrating approximately 1000 times per second. The similarity of the curves gave final evidence of the adequacy of the recording mechanism for studying the fundamental heart sounds.

IV. THE COMPONENTS OF THE FUNDAMENTAL HEART SOUNDS AND THEIR TIME RELATIONS

The sound vibrations responsible for the fundamental heart sounds heard on auscultation were recorded from different areas of the heart and large vessels in twenty-two dogs. In each case both the dynamic condition of the heart and the total peripheral resistance were varied experimentally in order to preclude the possibility that the recorded vibrations might not correspond to those found in the intact heart. Records were also obtained with recording capsules varying in their sensitivity and by communicating systems with different degrees of lateral opening. When sound vibrations from different cardiac regions were simultaneously recorded the capsules were repeatedly interchanged. These precautions were taken in order to discount possible unrecognized errors in the apparatus and technic. In this manner a large number of tracings were obtained in which the fundamental sound vibrations were compared with the dynamic and electrical changes in the heart. It is our purpose in this paper to analyze only what may be regarded as the normal ventricular and aortic sounds from the two sides of the heart.

1. The analysis of sound records. The vibrations assembled by the sound transmitter attached to the heart are a mixture of mechanical impacts and sound vibrations, but by appropriately adjusting the lateral opening to the sensitiveness of the recording capsule, a differential record of sound vibrations may be obtained. The accomplishment of this end involves the same principles and difficulties that are discussed by us (8) in the case of recording sounds from the thoracic wall. It is not always possible, therefore, to obtain records entirely free from mechanical oscillations while, in addition, extraneous, accidental vibrations of small amplitude may recur throughout the curve in spite of the best precautions. This may be illustrated by reference

to the illustrations accompanying this article. In the middle curve of figure 4, almost no extraneous vibrations or mechanical variations are introduced, the curve presenting a pure heart sound record. In the upper record mechanical variations are also absent, as indicated by the straight line between sounds. There are, however, a number of small accidental vibrations labelled x. In the records shown in figure 5, A and B, the curves are complicated by the additional presence of grosser mechanical variations which owe their existence to the fact that the lateral opening in the recording system was not quite adequate. Until it becomes technically possible to obviate these distortions in all cases the eye must be trained to distinguish the essential from the accidental and mechanical vibrations, much as the ear is trained in auscultation to disregard sounds for which it is not listening.

2. The components of the first ventricular sound. The vibrations recorded from different regions of the right and left ventricle show no essential differences (cf. figs. 5 A and 5 B); hence, they may be referred to in common as the *ventricular sounds.*

The first ventricular sound is composed of a series of five to thirteen irregular vibrations the periods of which range from 0.004 to 0.054 second. As their maximum period rarely exceeds 0.024 second, it is doubtful whether vibrations of longer period really may be classed as entering into the sound. The duration of the sound varies from 0.05 to 0.152 second (cf. illustrations and table 1). The components of the first sound are clearly discernible upon careful inspection of the various illustrations in spite of the fact that they were somewhat reduced in size during their reproduction. The sounds are shown in enlarged form in the upper record of figure 3. The sound starts with an *introductory vibration,* A. Occasionally there are two of these vibrations (cf. figs. 5 and 6). This is followed by a group of five or six *main vibrations,* B, the onset of which is always distinctly indicated by a sharp deflection of the light band, either in an upward or downward direction. They are followed by a group of *final vibrations,* C, varying in number.

Both the number and individual amplitudes of the vibrations in each group depend on the vigor of ventricular systole but their period is unaffected, as shown in the two segments of figure 4. Segment A shows the ventricular sounds recorded shortly after exposing the heart. In such cases the effective venous pressure and the arterial pressure are reduced; consequently, the heart is somewhat hypodynamic. Nevertheless, the sounds on auscultation are clear and dis-

TABLE 1

Ventricular sounds

EXPERIMENT	PLACE TAKEN	FIRST SOUND			SECOND SOUND			RATIO FIRST: SECOND SOUND DURATION
		Number of vibrations	Range in period	Duration	Number of vibrations	Period	Duration	
			sec.	*sec.*		*sec.*	*sec.*	*sec.*
C110	Right ventricle	5	0.0026–0.017	0.092	3	0.0115	0.028	3.2:1.0
C113	Right ventricle	6	0.004 –0.027	0.152	4	0.009	0.047	3.2:1.0
C115	Right ventricle	6	0.006 –0.010	0.050	3	0.007	0.015	3.3:1.0
C118	Right ventricle	5	0.006 –0.018	0.063	2	0.009	0.018	3.5:1.0
After adrenalin	Right ventricle	12–13	0.009 –0.018	0.126	3	0.009	0.027	4.7:1.0
After adrenalin	Right ventricle	10–11	0.006 –0.036	0.063	3	0.009	0.036	1.7:1.0
C120. After adrenalin	Right ventricle	12–13	0.006 –0.036	0.099	4	0.009	0.018	5.5:1.0
C122	Left ventricle	8–9	0.008 –0.024					
C123	Right ventricle	7–8	0.0062–0.0187	0.10	4	0.0125	0.056	1.7:1.0
C124	Left ventricle	7– 9	0.012 –0.018					
After adrenalin	Left ventricle	8–13	0.009 –0.024					
C125	Right ventricle	7– 9	0.007 –0.021	0.080	5–6	0.008–0.024(?)	0.044	1.8:1.0
C127	Left ventricle	7– 8	0.009 –0.054 (?)	0.068	3	0.013	0.025	2.7:1.0

TABLE 2

Aortic sounds

EXPERIMENT	PLACE TAKEN	FIRST SOUND			SECOND SOUND			RATIO FIRST: SECOND SOUND DURATION
		Number of vibrations	Range in period	Duration	Number of vibrations	Period	Duration	
			sec.	*sec.*		*sec.*	*sec.*	*sec.*
C111	Pulmonary	5– 6	0.012–0.018	0.058	3	0.020	0.035	1.6: 1.0
C112	Pulmonary	12–19	0.006–0.02	0.065				
C115	Aorta				7	0.007	0.045	
C116	Aorta	9	0.012–0.018	0.108	6	0.012	0.060	1.8: 1.0
C116	Pulmonary	7	0.006–0.018	0.048	10	0.009	0.042	1.1: 1.0
C118	Aorta	12–13	0.008–0.020	0.153	4	0.015	0.072	2.1: 1.0
After adrenalin	Aorta	9–10	0.008–0.018	0.126	4	0.018	0.063	2.0: 1.0
C120	Aorta	4– 5	0.009–0.018	0.028	2	0.009	0.018	1.5: 1.0
C125	Pulmonary	4– 5	0.007–0.013	0.039	2	0.007	0.021	1.8: 1.0
C127	Aorta	7– 8	0.009–0.018	0.081	2	0.018	0.036	2.2: 1.0

tinct and equal in intensity those normally heard over the chest wall. The recorded vibrations are few in number but comprise all the components of the first sound. They start with an *introductory rise*, A, followed by a group of four or five *main oscillations*, B, and a few final vibrations, C, discernible by very careful inspection. Segment B shows a similar series of sounds intensified in amplitude owing to the stimulating action of adrenalin on the heart. The introductory variation, A, is larger, the main oscillations, B, increase both in number and amplitude, and the final variations, C, become distinct. These three components which have been designated as the *introductory*, the *main* and the *final vibrations*, respectively, we consider characteristic of fundamental ventricular sounds. The introductory vibrations may be entirely lacking even during vigorous cardiac action and the final vibrations are wanting in essentially hypodynamic hearts. The main group of vibrations, though reduced in number of individual waves, is the last to persist in dying hearts.

A few words must be incorporated in regard to certain vibrations preceding the main group in phonocardiograms taken from the apex region of man. Fahr (1) described these variations and designated them the "initial variations." Their period is longer and they occur 0.02 to 0.04 second, exceptionally 0.09 to 0.10 second, before the main vibrations. Similar waves are recognized in tracings published by Bull (9), Battaerd (2) and in some of our unpublished tracings from men. We are in doubt whether these variations correspond to our introductory variations or to still another coarse variation sometimes preceding the introductory rise. Owing to this uncertainty we have employed the term "introductory" in our description to avoid possible confusion in the future. Of one fact we are certain—if the "initial vibrations" precede our "introductory variation" they are presystolic in time and have no claim to being a part of the first heart sound.

Fig. 3. Records of ventricular sounds (upper record) and aortic sounds (lower record) enlarged three times from original and showing details of sound vibrations. *A*, introductory vibrations; *B*, main vibrations; *C*, final vibrations in ventricular sound record; C_1, first component; C_2, second component of main vibrations in aortic record; *2S*, second sound.

Fig. 4. Two segments of a record showing aortic sounds (upper curve), right ventricular sounds (middle curve) and their relation to the electrocardiogram, Lead II. A, during hypodynamic heart action consequent to opening chest; B, during action of adrenalin (one-half actual size). *P*, relative alignment of points; *X*, accidental vibrations; S_1, first sound; S_2, second sound; *A*, introductory vibration; *B*, main vibrations; *C*, final vibrations of ventricular first sound; C_1, first component; C_2, second component of first aortic sound. Ink lines show synchronous points on three curves.

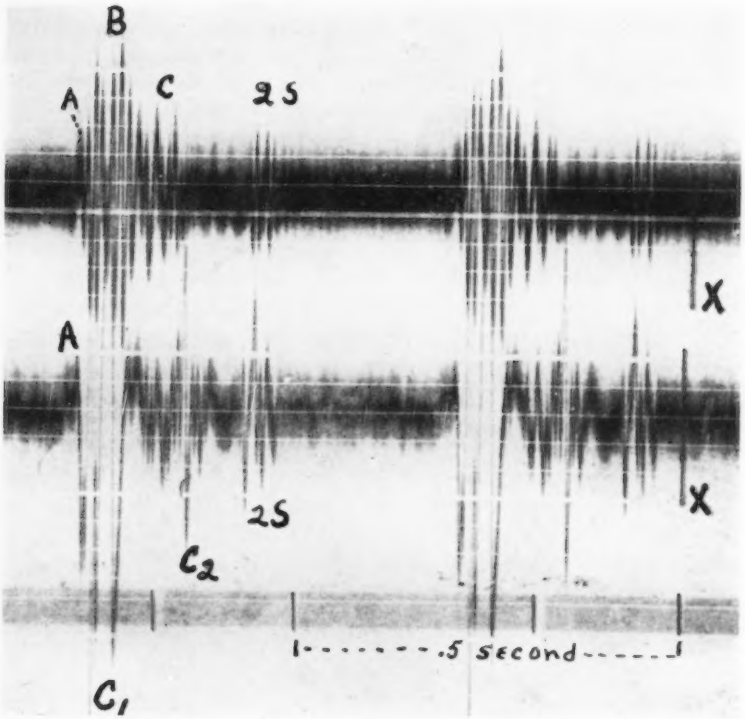

Fig. 3.

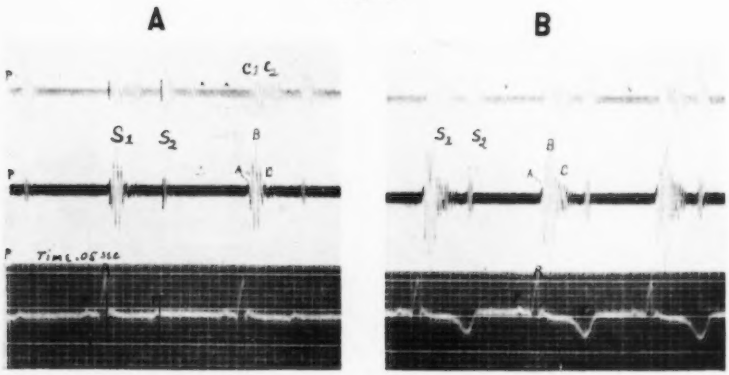

Fig. 4.

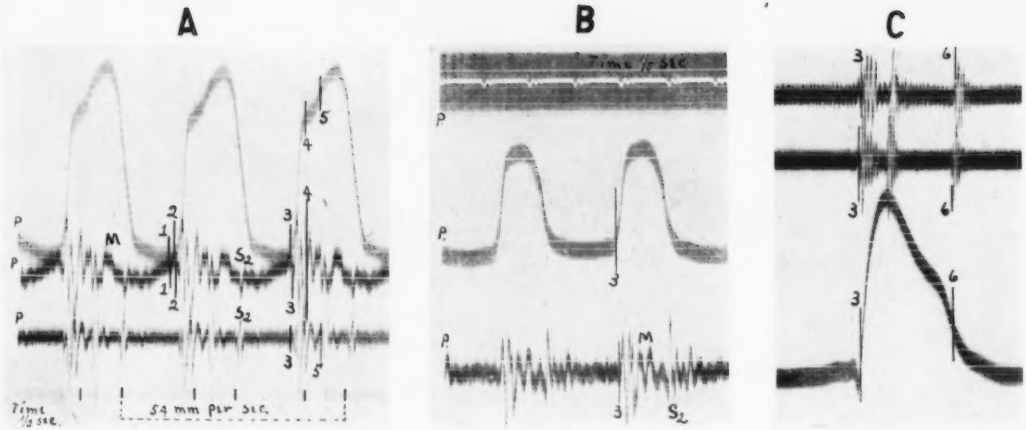

Fig. 5. Three segments of records from three different experiments showing relation of sounds to intraventricular pressure. (Three-fifths actual size.)

A. Left intraventricular pressure (upper curve), right ventricular sounds (middle curve), aortic sounds (lower curve). *P*, relative alignment of points; *M*, mechanical oscillations; S_2, second sound; Lines *1, 2, 3, 4* show corrected time relations of first sounds to intraventricular pressure curve.

B. Left intraventricular pressure and left ventricular sounds from apex of heart; letters same as before.

C. Aortic sounds (upper curve), pulmonary arterial sounds (middle curve), right intraventricular pressure (lower curve), showing two components of first sound in both aortic and pulmonary arterial curves; numbers same as before.

3. The relations of the components of the first ventricular sound to dynamic events. As the nature and number of introductory vibrations varies in different records of the first sound, it is of prime importance to determine what point may be taken as the beginning of the first sound. This question is of more than academic interest, involving, as it does, the relation of sounds to other events, and the point that may be taken as a criterion of the beginning of ventricular systole. These questions may be analyzed by a careful comparison of the three components of the first sound with the dynamic events of the cardiac cycle.

The relation of the first sound to the intraventricular pressure recorded by an optical manometer described by one of us is shown in the records of figure 5 taken from three different experiments. The relations are indicated by corresponding lines corrected for slight errors in the vertical alignment of apparatus. The *introductory vibration*, begins a trifle (average 0.018 second), but distinctly *before* the rise of intraventricular pressure (lines 1, 2). This is true of the right as well as the left ventricle. The *main vibrations* begin almost synchronously with the rise of intraventricular pressure (lines 3). They reach their greatest amplitude before the ejection period begins (lines 4). The *final vibrations* occur during the early portion of the ejection period. Recorded synchronously with the aortic pressure (fig. 6) it is found by measurement that the *introductory variations* precede the preliminary rise of arterial pressure described by Frank by 0.025 to 0.05 second (lines 1). The *main oscillations* begin with or very shortly after the rise of this preliminary variation (lines 3); i.e., during the isometric period of the ventricle.[3]

The vibrations attain their maximum amplitude (lines 4) just before the aortic pressure begins to rise, showing that they diminish rapidly as soon as ejection occurs. The *final vibrations* appear during the rise of the primary wave of aortic pressure but end shortly after its summit is reached (lines 5).

A comparison of the first sound with the right auricular myogram (fig. 7) shows that the introductory vibration begins a short interval (average 0.018 second) after the termination of auricular systole, as indicated by the apex of the auricular myogram.

Two facts are evident; first, that the introductory vibration or

[3] The exact relation of the introductory vibrations is often difficult to determine, owing to the small size of the preliminary vibration.

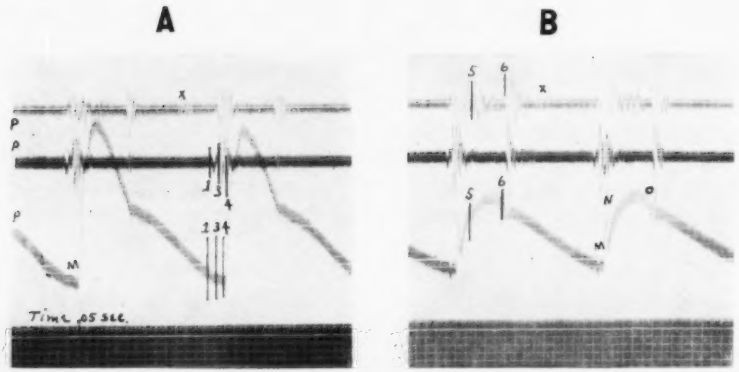

Fig. 6. Two segments of a record showing time relations of different components of the aortic sounds (upper curve) and right ventricular sounds (middle curve) to intra-aortic pressure (lower curve). (One-half actual size.) A, during hypodynamic cardiac action; B, after compression of aorta; other numerals and letters same as before. *M*, preliminary vibration; *N*, primary oscillation; *O*, incisura of aortic curve.

Fig. 7. Segment of record showing relation of first right ventricular sound to end of auricular systole, as indicated by apex of auricular myogram (upper curve); second sound accentuated. (About three-fourths actual size.)

vibrations occur during auricular relaxation and a variable, though short, interval before the rise of intraventricular pressure; and second, that the main vibrations, whether they begin with an upward or downward movement, coincide with the first rise of intraventricular pressure. Either one of two conclusions may be drawn: (1) That the ventricular muscle gives evidence of contraction by an introductory sound vibration before it is capable of elevating the intraventricular pressure; or (2) that the introductory sound vibration does not represent the onset of ventricular systole. We have no evidence enabling us to decide between these alternative conclusions. Inasmuch as the onset of the introductory vibrations is so variable and so frequently absent in records from the thoracic wall, and inasmuch as the onset of the main oscillations is so precisely related to a definite dynamic event; viz., the rise of intraventricular pressure, we propose to utilize the first of these main oscillations in time comparisons.

4. The relation of the first sound vibrations to the electrocardiogram, Lead II. The relation of the main vibrations of the thoracic sounds to the waves of the electrocardiogram have been frequently studied. Kahn (10), utilizing Weiss's phonoscope, found that these vibrations began during the descending limb of R_{II} or 0.01 second before its completion. Bull's results (9) are essentially in agreement. He found that the first sound began 0.03 to 0.04 second after the rise of the R wave; i.e., near its completion. According to Battaerd (2) the first sound follows the rise of R_{II} by 0.02 to 0.065 second, the interval apparently depending on the intensity of the sound and the sensitiveness of the microphone transformer. The results of Lewis (7) and Fahr (1) are in essential agreement but Fahr considers the initial vibration as indicative of the onset of ventricular systole, thereby placing this event on the ascending limb of R_{II} and occasionally, it must be confessed, even before the rise of R_{II} (cf. fig. 3 in his article). Records taken by Williams (personal communication) indicate that the onset of the main oscillations may begin near the R_{II} peak; i.e., 0.025 second after the onset of R_{II}.

A study of our records of the fundamental sounds (cf. fig. 4) shows that the introductory variations begin 0.01 to 0.02 second after the rise of R_{II}; i.e., on the ascending limb. The main oscillations, however, never begin before the peak of R_{II} has been reached. They occur from 0.03 to 0.045 second after the onset of R_{II}; i.e., on the descending limb or after its completion.

5. The characteristics of the second ventricular sound. The second

ventricular sound, when compared to the first, is composed of a much simpler set of vibrations (fig. 3), averaging two to six in number and lasting 0.015 to 0.056 second. The ratio to the duration of the first sound varies from 1 : 1.7 to 1: 5.5 (table 1). The vibrations rapidly reach a maximum and show a rapid decrement. Their number and amplitude depend on the vigor of ventricular systole (fig. 4) as well as on the height of diastolic pressure (fig. 6). Their period is quite constant in each case and varies from 0.007 to 0.0125 second in different animals, as shown in table 1.

The maximal amplitude of the second vibration, as a rule, is less than that of the corresponding first sound (figs. 3 and 4, A) but when arterial pressure is greatly increased, as during the action of adrenalin (fig. 4, B) or on compression of the aorta (fig. 6), it may very nearly equal that of the first sound. Upon auscultation a distinctly accentuated second sound is heard (cf. also fig. 7, in which an accentuation of the second sound was a natural occurrence).

In some cases the vibrations of the second sound are barely recognizable. This occurs in some cases when cardiac action, as indicated by the contour of the intraventricular curve, is apparently normal. This is the case in figure 5 A, when the second sound is hardly indicated in spite of considerable closure of the transmitting system. In such cases they become more distinct again after the administration of adrenalin.

6. The time relations of the second ventricular sound. Figures 5 A, B show clearly the relation of the second sound to the intraventricular pressure curve. The vibrations of the second sound begin an extremely short interval after the pressure begins to fall in the relaxation period. Comparison with the aortic pressure curve (fig. 6) confirm these relations. The first vibration of the second sound corresponds very nearly to the beginning of the incisura (lines 6). The vibrations are completed 0.015 to 0.025 second after the bottom of the incisura is reached. Comparison with intra-auricular pressure curves showing a fall of pressure early in ventricular diastole (fig. 8) indicates that the second sound precedes this fall by 0.06 to 0.08 second (lines 6, 7).

The relation of the second ventricular sound to the T wave was found to be extremely variable. Occasionally the onset corresponded with remarkable precision to the end of the T wave (figs. 4 A, 9). At other times it began before this wave had reached its end. When the T wave is negative, as after the administration of adrenalin, the

second sound may begin before the trough of the T wave is reached (fig. 4 B). This, it may be added, occurred in spite of a shortening of the R–T interval.

7. The components of the first aortic sound and their time relations. The first sound recorded directly from the aorta or pulmonary artery as a rule, contains more vibrations than the corresponding first ventricular sound. Its duration varies from 0.028 to 0.15 second (table 2). In some records the configuration of the first aortic sounds does not differ essentially from that of the first ventricular sounds but in most records essential differences exist which persist upon exchange of registering capsules (fig. 3). The aortic vibrations, like those from the ventricle, may or may not start with an *introductory variation*, A. It may be present in one record and not in the other when synchronously recorded. Hence, if we attempt time comparsions between the two sounds from the first recorded introductory variation discrepancies occur. Following the introductory variation we recognize a group of large oscillations that will be referred to as the *first component*, labeled C_1 in figures 3 and 4. These oscillations start precisely with the main vibrations of the first ventricular sound. The time relations established for the latter, therefore, also hold for the first aortic sound. The periods of this component do not correspond vibration for vibration with the ventricular sound (figs. 3, 4, 5 A, 6), nor is the maximum amplitude reached synchronously. As a rule, the frequency is greater and the maximum reached earlier in the aortic curves (fig. 3). Following these vibrations comes a *second component* (marked C_2 in figs. 3 and 4) which generally reach a second maximum amplitude during the ejection period. This is shown by their comparison with the intraventricular and aortic pressure curves (5 A and 6, lines 5). In some experiments the vibrations of the second component are continuous with those of the first component (fig. 4 B) but, owing to the fact that two distinct sets of large vibrations occur during the sound, they give it a configuration essentially different from the first ventricular sound. A peculiar variation of this component is found in some experiments; viz., that it may be apparently dissociated from the first component and occur as an isolated set of vibrations during midsystole (figs. 3, 5 A); in other words, the first sound is reduplicated. Occasionally it is displaced so late into systole and so near the second sound that the first impression is one of a reduplicated second sound, a fact rendered improbable by its time relations to dynamic events. Neither the cause nor the significance of this dissociation is clear.

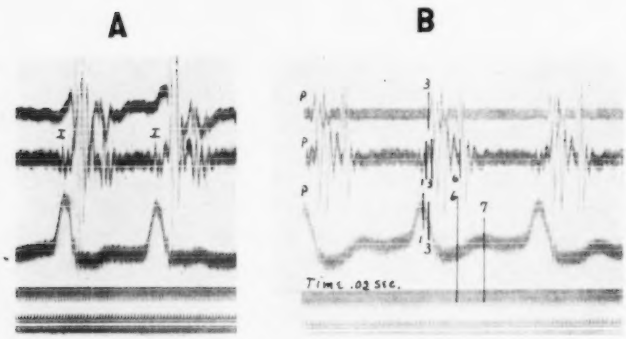

Fig. 8. Two segments of a record showing the time relation of the pulmonary sounds (upper curve) and aortic sounds (middle curve) to the right intra-auricular pressure (one-half acual size). Letters and numerals same as before; 7, diastolic fall of intra-auricular pressure following opening of tricuspid valve. First segment normal; second segment during depressed cardiac action due to pituitrin.

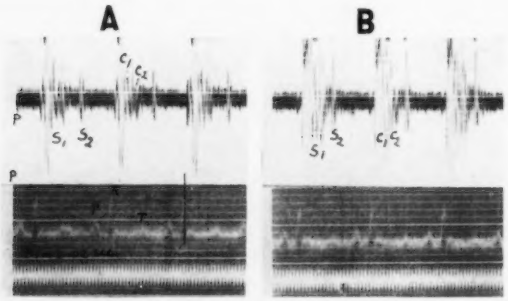

Fig. 9. Two segments of record showing effect of adrenalin (second segment) on components of first and second pulmonary sounds and time relations to electrocardiogram, Lead I. Lettering same as before. (Three-fifths actual size.)

The possibility must, of course, be borne in mind that in such cases the dissociation may be virtual and not real, that between these components small vibrations exist which are too feeble to record. The second component is frequently absent in records from the pulmonary artery but, again, may be as prominent as that of the aortic record (fig. 3 C). Of the influences which modify the vibrations of the first aortic sound a number may be noted. Following the administration of adrenalin, during asphyxia and after aortic compression the amplitudes of both components increase (figs. 4, 6, 9). The second component is entirely absent when arterial pressure is low (fig. 8, B). In such cases it may frequently become prominent upon compression of the aorta (fig. 6). In hypodynamic hearts the first component is smaller in amplitude than the second, giving the second component an accentuated appearance. Compression of the aorta and administration of adrenalin in such cases intensify the first component and reestablish the usual relations in amplitude.

8. The characteristics and time relations of the second aortic sound. The second aortic sound consists of a series of two to ten large decrescendo vibrations (fig. 3). Its duration varies from 0.018 to 0.072 second and is, therefore, considerably shorter than the first aortic sound, the ratios of the two sounds varying from 1: 1 to 2: 1 (table 2). Its onset coincides with the second ventricular sound, indicating the beginning of diastole, as shown by comparison with the intraventricular and arterial pressure curves (figs. 5 A, 6). The relation to the electrocardiogram is shown in figure 9.

The second aortic differs from the second ventricular sound in that the amplitude of its vibrations is larger than those of the second ventricular sound and usually exceeds those of the first aortic sound (figs. 3 and 4). The latter fact accords with auscultatory findings—that the second arterial is louder than the second ventricular sound. This relation is not always found, however. The first aortic sound may consist of vibrations greater in amplitude than those of the second sound. This is the case in figure 6. This variation can not be attributed solely to variations of blood pressure because the amplitude of the pulmonary sound frequently equals that of the aortic sound (fig. 5 C) and certainly the inequality of pressure in the two systems is evident.

SUMMARY

In order to investigate the nature, causes, variations and time relations of the heart sounds it is of greatest importance to record accurately

the fundamental vibrations that arise in the heart and large vessels. For a number of reasons it is questionable whether the heart sounds recorded from the thoracic wall are composed of vibrations identical with those occurring within the heart. We therefore devised a new form of sound transmitter which can be applied to any spot on the heart or large vessels and transmits the fundamental sound vibrations to a modified form of Frank's segment capsule. By simultaneously recording the pressure changes within the auricles, ventricles and aortae by an optical manometer, and the electrical variations of the heart by Williams's model of Einthoven's string galvanometer, it has been possible to relate precisely the fundamental sound vibrations to the dynamic and electrical events occurring during each cardiac cycle.

The first ventricular sound so recorded may be described as consisting of three continuous elements:

1. One or two *introductory vibrations* which begin during auricular relaxation, precede by a variable interval the onset of intraventricular pressure, and occur during the rise of the R_{II} wave of the electrocardiogram.

2. The *main vibrations*, composed of seven to thirteen vibrations of irregular period and amplitude which begin with the onset of the pressure rise within the ventricle, reach their maximum during the isometric period and begin on the descending limb of the R_{II} wave of the electrocardiogram.

3. Three to six *final vibrations* which occur during the period when the ejection of blood from the ventricle takes place and during the R–T interval of the electrocardiogram.

The first aortic and pulmonary sounds are also divisible into three components which do not correspond, however, in period, amplitude or grouping to those of the first ventricular sound and, consequently, give the curve a configuration essentially different from that of the first ventricular sound. The components are:

1. One or two *introductory vibrations* comparable to similar vibrations in the first ventricular sound.

2. The *"first component"* of main vibrations, consisting of a group of vibrations which begin synchronously with the main vibrations of the first ventricular sound and reach their maximum during the isometric period.

3. The *"second component"* of main vibrations, occasionally consecutive to the first component but often dissociated from it, so as to give the sound a reduplicated character. These vibrations occur during

the ejection period of the ventricle and reach a maximum amplitude during a variable portion of this period.

The second sounds from both the arteries and ventricles consist of a short, simple series of vibrations, occurring precisely at the onset of diastole, as evidenced by their occurrence at the beginning of the diastolic fall of intraventricular pressure and the onset of the aortic incisura. They precede the opening of the a–v valves by a considerable interval, as evidenced by their comparisons with the diastolic fall of pressure in the auricle. They may, but do not necessarily follow the T_{II} wave of the electrocardiogram. The second aortic and pulmonary sounds differ from the synchronously recorded second ventricular sounds by their larger amplitude and sometimes by the greater number of vibrations.

It would be possible, and perhaps permissible, to construct a hypothetical explanation as to the causes of these differences in the fundamental ventricular and aortic sounds. It seems preferable, however, to reserve such speculation until further experimental data are available.

BIBLIOGRAPHY

(1) Fahr: Heart, 1912, iv, 147.
(2) Battaerd: Heart, 1915, vi, 121.
(3) Wiggers: This Journal, 1916, xl, 218.
(4) Gerhartz: Registrierung des Herzschalles, Berlin, 1911, 62. Arch. f. d. gesammt. Physiol., 1910, cxxi, 530.
(5) Einthoven: Arch. f. d. gesammt. Physiol., 1907, cxx, 31.
(6) Frank and Hess: Verhandl. d. Kongress f. innere Med., 1908, xxvi, 285.
(7) Lewis: Heart, 1912–13, iv, 241.
(8) Wiggers and Dean: Amer. Journ. Med. Sci., (in press).
(9) Bull: Quart. Journ. Exper. Physiol., 1911, iv, 289.
(10) Kahn: Arch. f. d. gesammt. Physiol., 1909, cxxix, 291; 1910, cxxxiii, 597.

THE DEPRESSOR FUNCTION OF THE THORACIC SYMPATHETIC NERVE AND ITS CONNECTIONS

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THE VASCULARITY OF THE KIDNEYS AND INTESTINES DURING SPLANCHNIC DEPRESSION

Provided that currents of low intensity and frequency are employed, the stimulation of the intact thoracic sympathetic, greater splanchnic or abdominal sympathetic nerve, invariably leads to a very decisive fall in blood pressure. The same result may also be obtained by the excitation of the central end of one or the other of these nerves, and besides, this reaction does not disappear after the opposite thoracic sympathetic, both vagi and both phrenic nerves have been cut (1). My attention has recently been called to a preliminary notice of Auer and Meltzer (2) in which it is stated that the electrical stimulation of the central end of the splanchnic nerve causes an unmistakable drop in blood pressure. I am more than glad to permit my data pertaining to this point to serve in complete confirmation of the results of these authors.

The perseverance of the depressor action of the thoracic sympathetic nerve and its branches after the division of the nerves enumerated previously seems to show that this reaction is participated in by certain vascular areas which are not under the direct control of the splanchnic system. The deduction must therefore be made that the depression is not due to a local splanchnic reflex, but is dependent upon a vaso-dilatation of wide extent. This assumption, however, does not preclude the likelihood that the blood vessels of the splanchnic organs ordinarily take part in the depression, in fact, it has been pointed out by me that they do, because the division of the abdominal nerves diminishes the intensity of the reaction. In the succeeding pages a number of experiments are described which have a direct bearing upon this question.

As is true in the case of the depressor cordis, the circulation of the

abdominal organs may be affected in two ways, namely, actively and passively. Under ordinary conditions of experimentation, however, it is quite impossible to distinguish between these reactions, because they coincide in time and their general character is practically the same. Realizing this difficulty, the experiments to be described have been conducted in such a way as to enable me first of all to detect the latter type of changes. Their analysis has been attempted oncometrically as well as by the direct measurement of the blood supply of the kidneys and intestines. To begin with, however, they consisted merely in the registration of the volume of the kidneys while stimulation of the central end of one or the other thoracic sympathetic nerve was resorted to. The experiments were performed upon dogs, ether being used for the narcosis. The left femoral artery having been connected with a mercurial manometer, the abdomen was opened and the kidneys isolated sufficiently to be placed in oncometers later on. The diaphragm was then pierced in the region of the crura so that shielded electrodes could be applied to the central end of one of the thoracic sympathetic nerves. The stimulations were made at intervals, a Du Bois-Raymond coil and three-volt battery being made use of.

The possible criticism that the fall in blood pressure might be due to a local splanchnic reflex, I have sought to meet first of all by a complete isolation of the nerve by means of rubber membrane. Moreover, the strength of the current was always so slight that a direct stimulation of neighboring tissues could not have resulted. In many cases I have separated the nerve from its surroundings by dividing the lower thoracic roots so that the electrodes could be applied at least two inches away from any nervous connections. These details are in a way self-understood and I mention them merely to make certain of this point.

That the reaction is not confined to local vascular areas is also indicated by the general course of the blood pressure and its relation to the blood supply of the abdominal organs. In amplification of this statement attention is again called to the influence which the division of different nerves exerts upon this reaction. I might also mention that an even more complete isolation of the splanchnic organs has been attempted in several instances by cutting both thoracic sympathetic, both vagi and phrenic nerves and the rami connecting the lumbar ganglia with the spinal cord. Under this condition the excitation of the central end of the thoracic sympathetic nerve gave results such as are illustrated by the accompanying figure 1. Although it is evident that

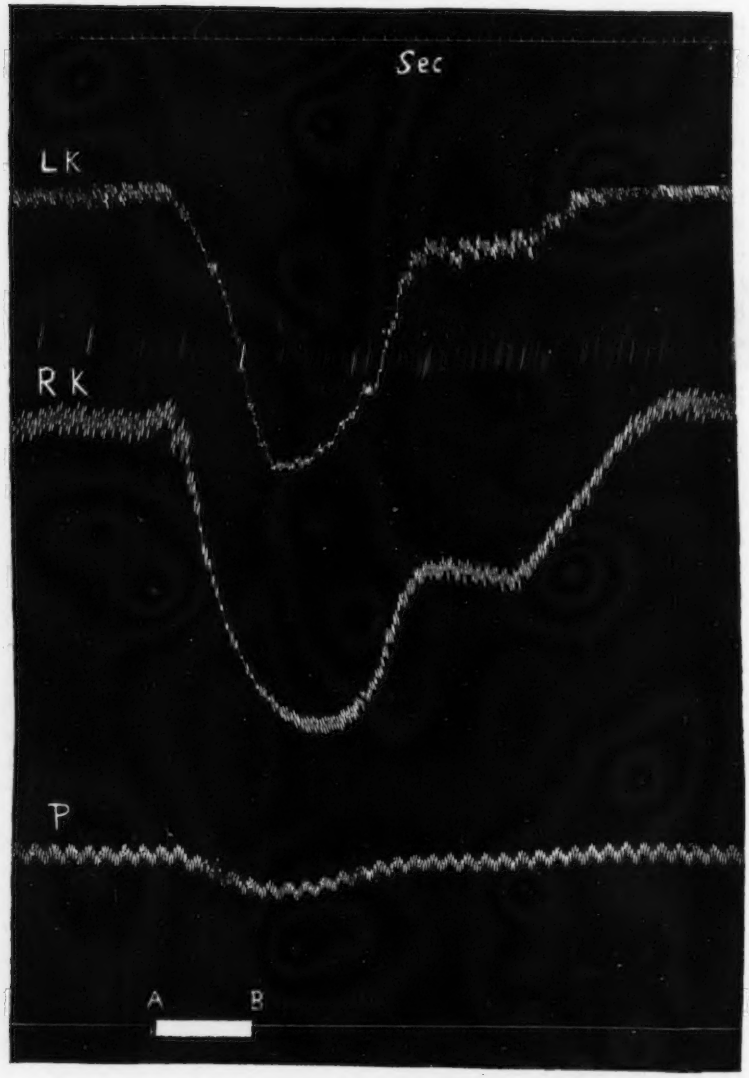

Fig. 1. Oncometric tracings of the kidneys (LK and RK) in relation with general blood pressure (P). Stimulation of the central end of the right thoracic sympathetic nerve.

the amplitude of the fall in the systemic blood pressure is somewhat diminished, its general outline is unchanged; indeed, a certain loss in its conspicuousness is to be expected, owing to the exclusion of the abdominal organs and the vascular relaxation following the division of the nerves enumerated.

As far as the vascularity of the kidneys is concerned it is apparent that it is wholly subservient to the arterial driving force. With the decline in the general pressure the volume of the kidneys decreases, while the subsequent reestablishment of normal values permits the oncometer record to attain the level previously occupied by it. The step-like adjustment of the latter, so clearly marked in this curve, corresponds to a similar accidental gradation of the blood pressure. It does not seem to have a deeper cause, because the division of the nerves precludes the possibility of an involvement of the adrenal bodies. This point, moreover, has been definitely settled by the extirpation of these organs, repeated again in the course of these experiments.

The measurements of the blood supply of the kidney by means of the stromuhr have led to results which closely agree with those just described. In illustration of this statement I insert in this place a limited number of phases which were obtained during the stimulation of the central end of the right thoracic sympathetic nerve, the left nerve, as well as all other nervous connections, having been left intact in this animal. The stromuhr (St) was inserted in the left renal vein. The pressure prevailing in this blood vessel, is indicated in figure 2 by the record V P, directly above the line of the signal marking the period of stimulation (A to B). To save space the general arterial blood pressure (AP) has been adjusted to the same zero line as the venous pressure. The data presented in the succeeding table are directly transferable to the figure.

A comparison of the values of the blood flow obtained during the stimulation with those immediately preceding and following this period, shows very clearly that the drop in the blood pressure is associated with a pronounced reduction in the blood supply of the kidney. Thus, we observe in this particular case that the normal flow of 2.59 cc. in a second is reduced, subsequent to point *A*, to 1.60 cc. in a second. It is also evident that the flow gradually increases after the cessation of the stimulation; in fact, the readjustment frequently appears even before the excitation has been completed.

These results quite agree with those obtained with the aid of the oncometer. Obviously, therefore, the division of the nerves enumerated

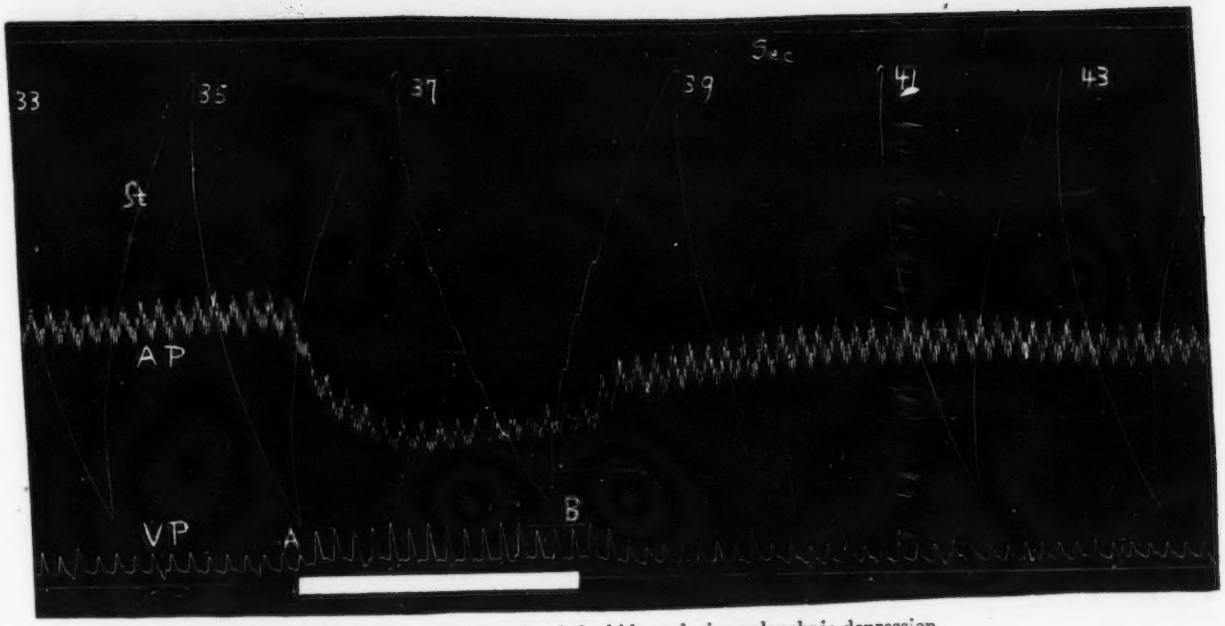

Fig. 2. The blood supply of the kidney during splanchnic depression

previously, brings it about that the kidneys meet the fall in the blood pressure in a perfectly passive manner. A slightly different picture however, is obtained, if one or both thoracic sympathetic nerves are left intact. The stimulation of one or the other of these nerves then induces a fall in the pressure which, to begin with, is characterized by a certain active resistance on the part of these organs. Very shortly, however, the loss in the arterial driving force renders this dilator attempt useless and the volume of the kidney decreases as it did when both

TABLE 1

Blood supply of the left kidney

NUMBER OF PHASE	TOTAL QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD FLOW	BLOOD PRESSURE ARTERIAL	PROCEDURE
	cc.	*sec.*	*cc./sec.*	*mm. Hg.*	
29	22.4	9.2	2.43	102.5	None
30	22.8	9.1	2.50		
31	22.8	9.0	2.53		
32	22.5	9.0	2.50		
33	22.6	9.2	2.45	103.5	
34	22.0	9.3	2.36		
35	23.1	8.9	2.59	108.1	
36	23.1	9.8	2.35	67.8	Stimulation: central end, right thoracic sympathetic nerve, 25 cm., 26 seconds
37	21.8	13.6	1.60	53.0	
38	21.8	12.3	1.77	76.0	
39	23.0	9.8	2.34	82.2	
40	23.2	9.0	2.56		
41	21.7	8.1	2.67	92.5	
42	21.8	8.5	2.56	96.6	
43	22.8	8.5	2.68		
44	22.8	8.8	2.59	89.5	
45	21.0	8.6	2.44		
46	21.0	8.5	2.47	102.6	

nerves were divided. This activity on the part of the kidney betrayed itself in the curves by a slight rise in the venous pressure coincident with the early phase of the fall in pressure. The most probable explanation of this lies in a temporary augmentation of the flow through this organ occasioned by a dilation of the renal blood vessels. In general, however, it holds true that a fall in blood pressure and a diminution in the blood supply of the kidney result (a) when the intact right or left sympathetic chain is stimulated, (b) when only one of these nerves is left intact and the central end of the other is subjected to the excita-

tion and (c) when under precisely the same condition the vagi, phrenic and lumbar nerves are divided in addition to the sympathetic.

It has been brought out quite clearly by Bradford (3) that the stimulation of the distal end of the splanchnic nerve with currents of low frequency and strength gives rise to a slight fall in blood pressure which is accompanied by a shrinkage of the kidney. It is concluded by this author that this result is dependent upon a peripheral effect and not upon a reflex excitation, and hence, it must be attained with the help of certain efferent components of this nerve. It is also assumed that this shrinkage of the kidney is a passive phenomenon, made possible by dilatations in other parts of the body and especially in the intestines. Obviously, therefore, the latter organ must act as a compensatory vascular area, the relaxation of which causes a withdrawal of blood from the renal circuits and a loss in the volume of these organs.

In order to test the validity of this statement, I repeated the experiments described previously after the mesenteric arteries and veins had been ligated. As the results differ in no way from those obtained before the exclusion of the intestines, the inference seems justified that the shrinkage of the kidney is not necessarily dependent upon a dilation of the intestinal blood vessels. In fact, in accordance with the preceding statement that the fall in blood pressure persists even after the division of both thoracic sympathetic, vagi and lumbar nerves, the conclusion must be drawn that the dilatation also affects certain blood vessels which are not innervated by the splanchnic system. This reaction, therefore, is brought about reflexly through the intervention of the spinal cord and higher centers and is not due to a direct peripheral effect in the sense of Bradford. Owing to the mode of preparation, this conclusion is self evident.

In the nature of things, the only local reflex to which the reactions just mentioned could be ascribed, would be a vaso-dilation in the realm of the splanchnic organs. We have seen that the kidney is not affected in this way, and even if the blood vessels of this organ did attempt to dilate, its shrinkage is sufficient proof of the fact that the fall in the systemic pressure quite offsets the dilatation. In the second place, the results of the exclusion of the intestinal blood vessels tend to show that the volumetric decrease of the kidney is not accomplished by a compensatory transfer of blood into the vascular channels of the intestines.

This fact I have sought to establish more thoroughly by comparing the blood flow through the intestines before the excitation of the cen-

tral end of the thoracic sympathetic nerve with that prevailing during the stimulation. The experimental procedure here followed was the same as that made use of in the case of the kidney. It should be mentioned, however, that the stromuhr (4) was inserted in the mesenteric vein at its junction with the vena gastro-lienalis. The venous pressure was recorded by a membrane manometer which communicated with the distal cannula of the stromuhr.

With the exception of the greater volume of the flow, these records

TABLE 2

Blood supply of the intestines

NUMBER OF PHASE	TOTAL QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD FLOW	BLOOD PRESSURE ARTERIAL	PROCEDURE
	cc.	*sec.*	*cc./sec.*	*mm. Hg.*	
9	20.8	6.0	3.46	95.4	None
10	20.5	6.4	3.20		
11	20.6	6.6	3.12		
12	20.4	5.8	3.51	97.0	
13	21.4	6.0	3.56		
14	21.4	6.4	3.32		
15	20.7	6.0	3.45	96.5	
16	20.5	14.0	1.46	54.9	Stimulation: central end, right thoracic sympathetic nerve, 25 cm., 20 seconds
17	20.3	13.0	1.56	50.1	
18	20.3	9.6	2.11	58.8	
19	21.0	9.0	2.33	80.0	
20	20.0	7.6	2.63		
21	21.2	7.2	2.94	86.4	
22	21.2	6.4	3.47	98.0	
23	20.5	6.0	3.41		
24	21.0	6.2	3.38		
25	21.0	5.9	3.55	97.5	
26	20.6	6.2	3.32		

agree perfectly with the one portrayed in figure 2. For this reason, it may suffice to insert at this time merely the values for a limited number of phases recorded during one of the stimulations. The principal fact to be gathered from this table is that the vascularity of the intestine decreases steadily with the blood pressure, the minimal flow coinciding with the lowest pressure. Obviously, therefore, the shrinkage of the kidneys is a parallel reaction and cannot be attributed to a dilation of the intestinal blood vessels. By inference it may be surmised that the other abdominal organs are affected in precisely the same way. Con-

sequently, it seems that a compensation, such as has been assumed by Bradford, does not play a part in the production of this depressor reaction.

THE FUNCTION OF THE MINOR SPLANCHNIC NERVES

Asp (5) states that the stimulation of the central and peripheral ends of the divided minor splanchnic nerve leads to an increase in the blood pressure and a diminution in the frequency of the heart. These results, as will be brought out later, are not in conformity with mine; moreover, as Asp uses the singular form in his reference to the minor splanchnic nerves, it is quite probable that the wide extent of the sympathetic

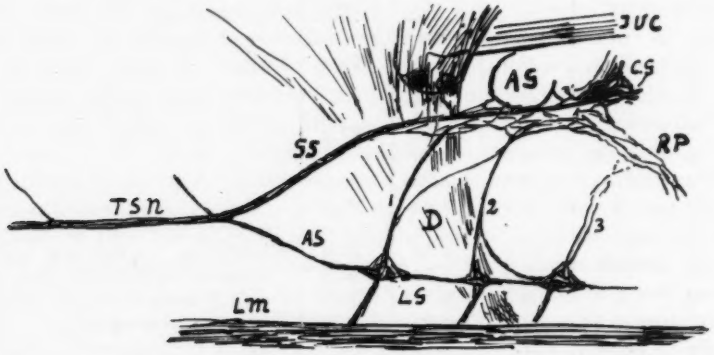

Fig. 3. *Lumbar Sympathetic System. TSN*, thoracic sympathetic nerve; *GS*, greater splanchnic nerve; *AS*, abdominal sympathetic nerve; *LS*, lumbar ganglia; 1, 2 and 3 minor splanchnic nerves; *RP*, renal plexus; *CG*, coeliac ganglion; *AG*, adrenal body and plexus; *IVC*, Inferior vena cava; *D*, edge of diaphragm and resected supra-renal blood vessels; *LM*, lumbar muscles.

network of this locality had not been recognized as yet, or that he mistakes the abdominal sympathetic for the first minor. Although certain variations are present, the relatively slender abdominal sympathetic nerve most generally leaves the greater splanchnic nerve at an acute angle, and while the latter extends forward and inwards to attain the region of the supra-renal glands, the former pursues a deep course along the lumbar vertebrae, where it unites with the different spinal rami of this region. A number of ganglia are formed at these points of union, from the first three of which the minor splanchnic nerves arise. This

entire system is commonly designated as the lumbar sympathetic system.

Near their places of origin the minor splanchnic nerves do not branch much and pursue a rather straight course towards the adrenal body, where they are connected by extensive ramifications with fibers of the greater splanchnic nerve. A relatively thick fiber of the latter generally continues right through this network and may be traced directly to the more distant solar ganglia. The minor splanchnic nerves, on the other hand, seem to tend largely towards the kidneys, some of their terminal fibers being directly traceable to this organ. The first and second minor nerves are frequently connected with one another by a fiber of considerable size; the second and third also show collaterals near their respective ganglia. An idea regarding the general course of the different fibers may be obtained from the neighboring sketch which gives the relationship existing on the right side of the body. To bring these nerves into view, the right kidney and liver must be pushed aside and the supra-renal blood vessels divided peripherally to the adrenal body. In a large dog the different minor splanchnic nerves may be isolated sufficiently to permit the application of the electrodes not only to the intact nerves, but also to their central or distal ends.

To begin with, the minor splanchnic nerves as well as their lumbar and thoracic connections, were left intact. The kidney of the corresponding side was placed in an oncometer, the recorder of which was adjusted in the same ordinate as the manometer registering the general blood pressure. Weak tetanic currents invariably produced a fall in blood pressure which under favorable conditions amounted to about 30 per cent of the normal pressure. The most conspicuous results were obtained with the first and second minors; moreover, these vascular depressions persisted even after the thoracic sympathetic nerve of the same side had been divided. On increasing the strength of the current, say from 25 cm. to 15 cm. distance of coils, the fall in blood pressure eventually gave way to a rise. It must be emphasized, however, that these reactions at no time attained the conspicuousness of those obtained with the help of the greater splanchnic or thoracic sympathetic nerve.

The minor splanchnic nerves were then divided about midway between the lumbar ganglia and the supra-renal plexus. The stimulations of the central end of any one of these nerves led to very characteristic depressions of the blood pressure, the amplitude of the reaction being greatest when the first minor was used and least when the third

nerve was employed (fig. 4). Moreover, while the general character of the reaction could not be changed by increasing the strength of the current from 25 cm. to 15 cm. distance of coils, the resulting reflex muscular efforts tended to raise the pressure, at least at the beginning of the excitation. Curare was not employed, however, because the depressor character of these nerves could easily be established with currents

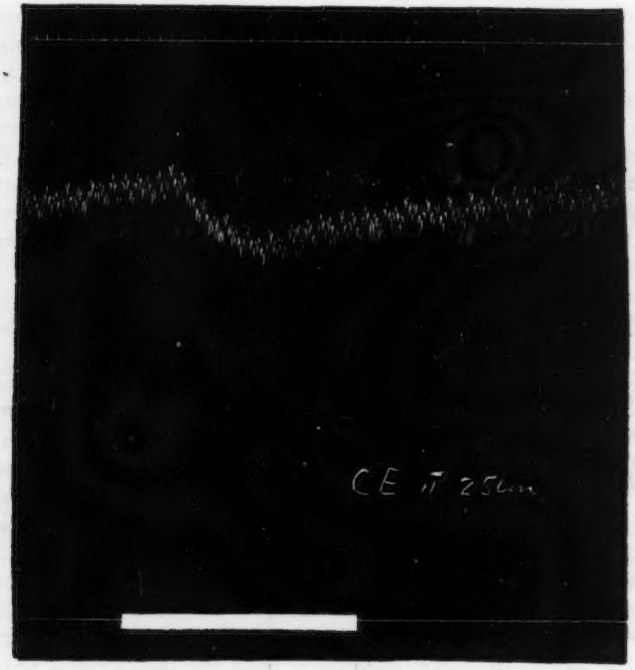

Fig. 4. Record of blood pressure during stimulation of the central end of the second minor splanchnic nerve.

of such slight intensity that perceptible muscular contractions did not result.

These stimulations were repeated after the division of the thoracic sympathetic and vagi nerves without that a change in the character of the reaction could be noted. The cutting of the lumbar roots, however, rendered the stimulations ineffective immediately. These results

clearly show that the minor splanchnic nerves conduct impulses in an afferent direction and that central parts may be attained by the latter by way of the radicles of the lumbar sympathetic ganglia. In the second place, it must be evident that the depressor action of these nerves is not dependent upon a local splanchnic reflex, but is brought about with the aid of central paths and nuclei. It is therefore a general reaction. For this reason, it cannot be regarded as similar to that of the

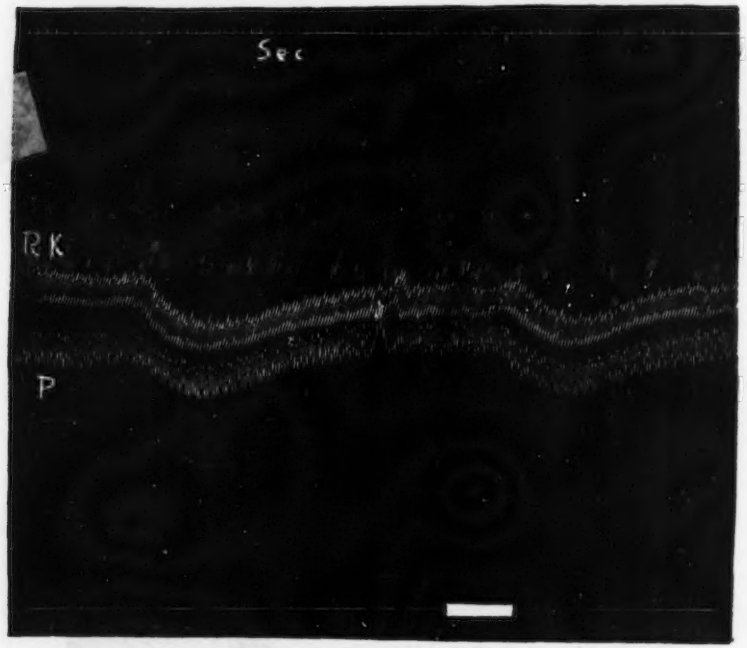

Fig. 5. General blood pressure (P) and the volume of kidney (RK) during stimulation of the central end of the first minor splanchnic nerve.

kidney, described by Bradford as being elicited by the excitation of certain posterior roots of this locality.

In following up this point more closely a number of oncometric records were obtained from the right kidney while the central end of the first or second minor splanchnic nerve of the same side was being stimulated. The thoracic sympathetic and vagi nerves were left intact at first but were divided subsequently. The fall in blood pressure was as-

sociated in all these cases with a diminution of the volume of the kidney; a complete passiveness of this organ, however, could only be noted after all the aforesaid nerves had been divided. A curve of the latter kind is represented in figure 5; the upper record is that of the oncometer and the lower that of the mercurial manometer. The first depression resulted in consequence of the mere adjustment of the electrodes to the

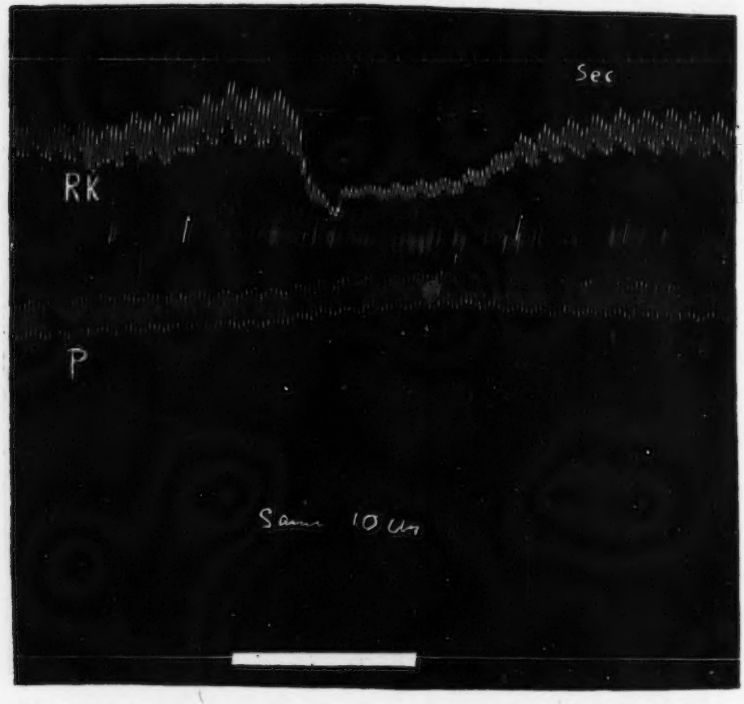

Fig. 6. General blood pressure (P) and the volume of the kidney (RK) during stimulation of the distal end of the second minor splanchnic nerve.

central end of the divided first minor splanchnic nerve and the second in consequence of its excitation with a weak current of eight seconds duration. The parallelism between these two curves is complete, and hence, it may justly be concluded that this depressor reaction may be brought about without the aid of the kidneys. In fact, as the depression is also obtainable after the division of the nerves previously men-

tioned, the splanchnic organs need not be actively implicated in this dilator process.

When tetanic currents of moderate strength were employed, the excitation of the distal end of any one of the minor splanchnic nerves resulted in a slight increase in the blood pressure. The most decisive results were obtained with the first and second nerves. A joint record of the blood pressure (P) and the volume of the kidney (RK) is given in figure 6. It may be gathered from this curve that the rise in the pressure is due to a constriction of the renal blood vessels of the same side, because if a larger vascular area were actually involved, this reaction would possess a much greater amplitude. This conclusion is borne out further by the fact that the shrinkage of the kidney continued in most cases for some time after the blood pressure had returned to its normal level. Thus, while the initial vaso-constriction in this organ tends to heighten the general blood pressure, the field affected is so small that a compensatory adjustment of the latter can readily be brought about.

BIBLIOGRAPHY

(1) Burton-Opitz: This Journal, 1916, xli, 103.
(2) Auer and Meltzer: Zentralbl. f. Physiol., 1913, xxvi, 1316; and Proc. Amer. Physiol. Soc., 1913.
(3) Bradford: Journ. Physiol., 1889, x, 358.
(4) Burton-Opitz: Pflüger's Arch., 1908, cxxi, 150.
(5) Asp: Arb. aus d. physiol., Anst. zu Leipzig, 1868, 131.